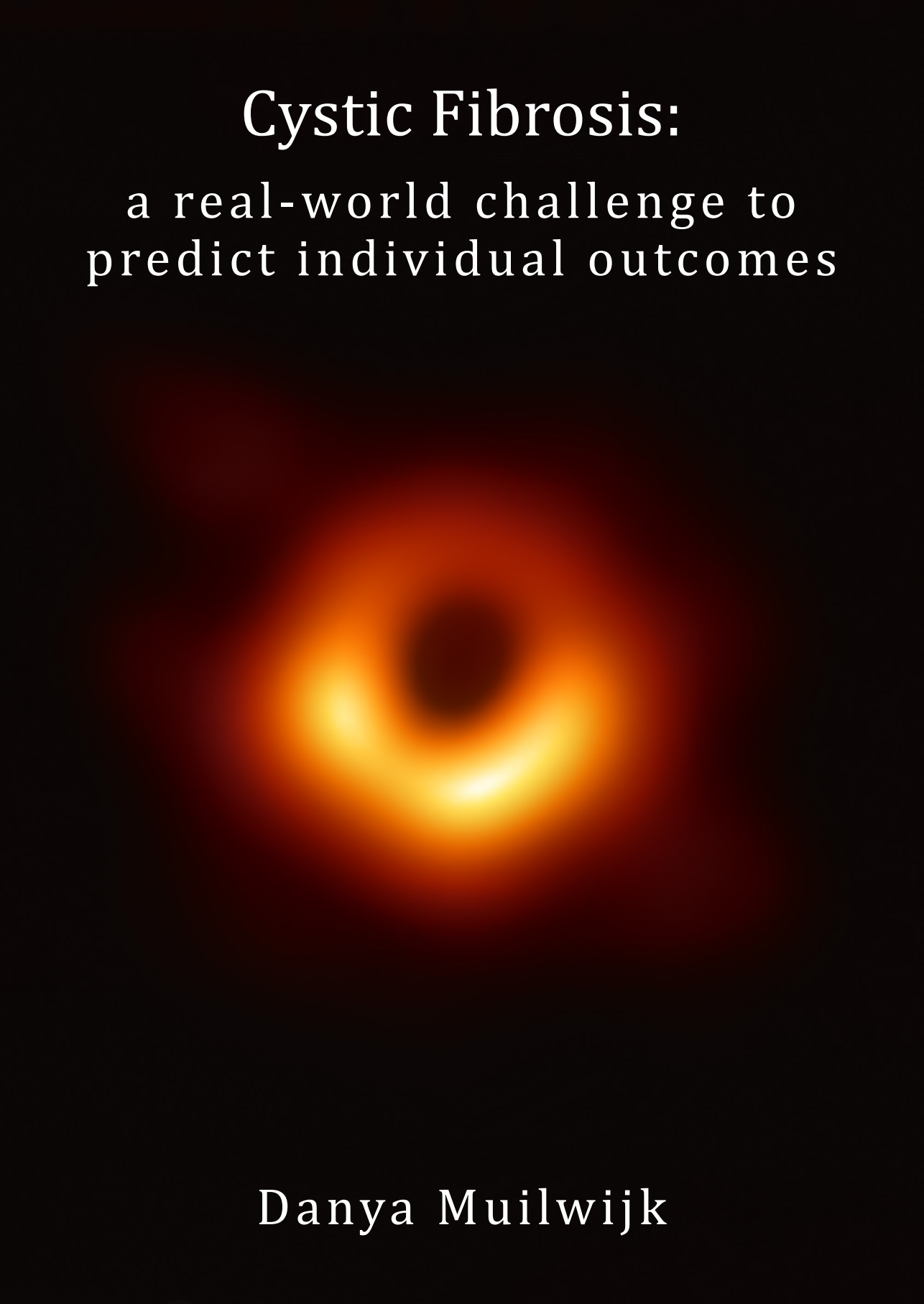
Cystic Fibrosis:

a real-world challenge to predict individual outcomes



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Colofon
Cystic Fibrosis: a real-world challenge to predict individual outcomes
PhD thesis, Utrecht University, The Netherlands
ISBN/EAN: 978-94-6419-928-4

(Cover) Layout: Ilse Modder
Printed by: Gildeprint, Enschede, The Netherlands
Credit cover image: ESO/EHT collaboration (https://eventhorizontelescope.org/)

Cystic Fibrosis: a real-world challenge to predict individual outcomes

Cystic Fibrosis: een ware uitdaging om individuele uitkomsten te voorspellen

(met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor aan de Universiteit Utrecht
op gezag van de rector magnificus, prof. dr. H.R.B.M. Kummeling,
ingevolge het besluit van het college voor promoties in het openbaar te
verdedigen op

vrijdag 27 oktober 2023 des middags te 12.15 uur

door

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geboren op 13 augustus 1988
te Dordrecht

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CONTENT

CHAPTER 1

General introduction

SUMMARY **for busy committee members and other rapid readers**

Next-generation health technologies and innovative methodological approaches are opening up exciting opportunities to leave the beaten tracks of traditional evidence-based medicine and propel further advancements in translational and personalized medicine [1]. Utilization of real-world data sources, integration of biomarkers and a transition towards more meaningful, patient-centered endpoints can enhance the drug development process and improve patient outcomes, especially in rare heterogeneous diseases like Cystic Fibrosis (CF) [1,2]. Over time, CF has become a model for harmonization of research development and clinical advancement in rare diseases, wherein understanding of pathophysiology and cellular biology is successfully translated into therapeutic innovations directly linked to improvement in patient care and survival [3].

Small molecules that target the underlying defect of CF have recently provided unprecedented progress in the treatment and prognosis of people with CF (pwCF) [4–8]. Nevertheless, additional work is still needed, as individual short-term responses to these cystic fibrosis transmembrane conductance regulator (CFTR) modulating drugs have shown a high individual variability which is difficult to predict [5–14]. Moreover, reimbursement and access to these therapies for eligible pwCF is still scattered across the globe due to high costs and healthcare burden [15,16]. Importantly, an urgent unmet need also remains for ~20-30% of pwCF who carry CFTR mutations which are not eligible for CFTR modulator therapy or rare CFTR mutations of which natural disease course and responsiveness to CFTR modulator treatment is unknown.

This thesis will focus on the utility of long-term real-world data for the development of new biomarkers such as patient-derived intestinal organoids and on personalized patient-reported outcome measures, which can help to move forward towards a cure for all individuals CF.

INTRODUCTION

Cutting-edge technologies and methodological advances are paving the way for a transformation in evidence-based medicine, which can help to overcome drug development challenges in rare heterogeneous diseases such as Cystic Fibrosis (CF) [1,2]. A vast source of real-world data, innovative biomarkers and meaningful patient-reported outcome measures (PROMs) could yield alternative or surrogate endpoints that may enhance the development and clinical translation of novel and personalized therapies needed to move forward to a cure for all people with CF (pwCF) [1,2,17].

CYSTIC FIBROSIS

The last decades have fostered tremendous scientific breakthroughs in the field of CF, a rare autosomal recessive monogenic disorder. Since the discovery of the *cystic fibrosis transmembrane conductance regulator* (CFTR) gene and the most common F508del mutation in 1989 [18–20], over 2000 CFTR mutations have been identified which differentially affect CFTR protein function and clinical phenotype (https://cftr2.org). The most prevalent mutations have been categorized into distinct classes according to the mechanism by which CFTR protein function is compromised or rescued [21–25]. In CF, the disrupted chloride and concomitant electrolyte and fluid transport across epithelial cells leads to accumulation of thick mucus in multiple organs. Although respiratory and digestive symptoms have always been the major causes of morbidity and mortality, CF encompasses a broad and heterogeneous spectrum of clinical disease manifestations. The extent of organ involvement, disease severity and degree of progression differs substantially among individuals.

Approximately 100,000 people are currently living with CF, and global prevalence is continuing to increase due to an ongoing improvement in life expectancy, along with an expanding identification of pwCF and growing data coverage in CF patient registries [26]. Initial improvements in survival were related to the advent of symptomatic therapies such as nutritional supplementation and pancreatic enzyme replacement [27], airway clearance therapies [28], mucolytics [29], long-term antimicrobial treatment to suppress airway infection [30–34] and lung transplantation [35], together with improved diagnosis through newborn screening programs [36,37] and centralization of specialized CF care [38].

CFTR modulating therapies

More recently, the emergence of CFTR modulators has further revolutionized the treatment landscape of CF, and accumulating data forecast an important contribution to bridging the survival gap between pwCF and the general population [39,40]. CFTR modulators are small molecules aimed to restore CFTR function by targeting the underlying protein defect at different levels. As a first in the field, ivacaftor (IVA) was developed to potentiate CFTR protein function by augmenting its channel opening probability. In 2011, IVA was proven efficacious as monotherapy in small subgroups of pwCF with at least one G551D [4] and other so-called gating mutations, which are carried by ~5% of the total CF population [41]. Subsequently, CFTR-correctors lumacaftor (LUM) and tezacaftor (TEZ) were designed to improve the defective mechanism of CFTR protein folding and trafficking to the apical cell surface, as caused by the most common F508del mutation. Together with IVA, these correctors formed the first dual CFTR modulator therapies LUM/IVA and TEZ/IVA, which got approved for pwCF homozygous for the F508del mutation in 2015 and 2017, respectively [5,6]. Addition of the latest new compound elexacaftor, which has both CFTR-correcting and potentiating properties [42], gave rise to the first triple combination elexacaftor/tezacaftor/ivacaftor (ETI), shown to be highly effective for pwCF who are homozygous or heterozygous for F508del [7,8,43].

Unmet need

This indicates that approximately 70–80% of all people with CF who carry at least one F508del mutation are nowadays eligible for highly effective CFTR modulator therapy, depending on age and prevalence of the mutation across geographic regions [44–46]. These drugs, however, come with a price, which has delayed or obstructed reimbursement around the globe, especially putting people from low-income countries at a disadvantage [15,16]. Moreover, treatment responses vary substantially between individuals and are difficult to predict [5–14]. Importantly, additional effective and personalized treatments are urgently needed for the remaining ~20–30% of pwCF who carry rare or ultra-rare CFTR mutations with unknown or absent responsiveness to CFTR modulators. As CF disease severity and long-term disease progression may vary across genotypes, biological and clinical characterization of these rare mutations and their responsiveness to treatments is important to identify individuals who may exhibit a more severe long-term disease courses and could benefit from new therapies.

CLINICAL TRIALS

Clinical trials with orphan drugs face challenges inherent to the rare disease population [2]. Participant pools are usually limited and restricted by rigid in- and exclusion criteria, based on theoretical, ethical, financial, practical and regulatory considerations. Furthermore, selection of appropriate endpoints that are both sensitive and relevant can be difficult due to high individual variability, which is especially problematic when estimated effect sizes of the intervention are small [2]. Selective trial conditions generally maximize the probability of trial success while mitigating the risk of financial losses and preventing harm, but may also hamper the translation of trial results into real-world practice of heterogeneous diseases such as CF.

Trial endpoints

As pulmonary symptoms have always been the major cause of morbidity and mortality [44,47], forced expiratory volume in 1 s (FEV1) is the most frequently used FDA- and EMA-approved primary endpoint in CF clinical trials. Notably, within-test variability and daily repeatability of FEV1 has been reported to be ~5% in adults with respiratory diseases including CF [48,49], up to a week-to-week and year-to-year variability of even 12–15% [50]. In sufficiently powered trials, FEV1 can be sensitive to detect small treatment effects on a group level, but the large intrinsic variability limits its sensitivity in smaller or heterogeneous study populations as well as in specific subgroups such as pwCF with mild or end-stage lung disease and children. In the context of the individual, it also complicates the detection of a significant and clinically meaningful change, which has to exceed natural variability.

Alternatively, a reduction in pulmonary exacerbation rate is frequently used as a trial endpoint, which reflects pulmonary inflammation and is associated with long-term lung function decline and survival [51–56]. Capturing a treatment benefit on pulmonary exacerbations, however, usually requires a relatively long follow-up period, which increases trial expenses, participant burden and the risk of drop-outs.

Beyond the pulmonary domain, sweat chloride concentration (SwCl) is often included as secondary endpoint, since it is regarded as a biomarker for CFTR function. Nevertheless, the sweat test is also subject to substantial biological, technical and environmental variability [57–60] and requires further validation to what extent measured changes reflect individual treatment benefit [61]. In addition, the respiratory symptom score of the Cystic Fibrosis Questionnaire-Revised (CFQ-R) is a commonly used secondary endpoint to determine changes in

disease-specific quality of life. It is the best validated subdomain score of the CFQ-R [62,63], but it is debatable whether respiratory symptoms actually reflect quality of life for pwCF. Furthermore, its sensitivity is expected to diminish in the changing CF population, as most pwCF are experiencing less respiratory symptoms under highly effective CFTR modulator treatment.

Short-term efficacy of dual CFTR modulators

The first phase 3 pivotal trials of LUM/IVA showed an improvement in the primary endpoint FEV1 of 2.4–4.0% after 24 weeks of treatment in pwCF homozygous for F508del who were older than 12 years of age with a baseline FEV1 between 40% and 90% [5]. This effect was only modest compared to the groundbreaking IVA trials, which showed an absolute change from baseline FEV1 of 7.5–10.4% after 8 to 24 weeks of treatment in people with CFTR-gating mutations [4,41]. Phase 2 and 3 LUM/IVA trials also disclosed a high inter-individual variability of short-term FEV1 responses in pwCF homozygous for F508del ranging from -10% to +10% [5,9] and failed to detect group-level efficacy of LUM/IVA in pwCF heterozygous for the F508del mutation in phase 2 studies [64]. Similarly designed RCTs with TEZ/IVA subsequently showed a comparable short-term efficacy and individual variability, albeit with a favorable side effect profile compared to LUM/IVA [6,65]. In addition, pwCF treated with one of the dual CFTR modulators showed a 30–39% reduction in PEx rate compared to controls, a 39–61% reduced rate of PEx leading to hospitalization and a 45–56% lower rate of PEx requiring intravenous (IV) antibiotics.

Long-term efficacy and real-world outcomes

Although short-term efficacy has been established for all CFTR modulators in designated subgroups, long-term evidence is only beginning to take shape given the relatively limited time period of their availability. The open-label extension trials of LUM/IVA and TEZ/IVA demonstrated an estimated FEV1 decline between -1.3% and -0.8% per year after 120 weeks of dual CFTR modulator treatment, compared to -2.3% to -2.1% in matched historical controls [66,67].

Several short-term real-world studies with a follow-up period ranging from 16 weeks up to 1 year after CFTR modulator initiation already suggested that the effectiveness of dual CFTR-modulators may be less strong than in trials and confirmed individual variability of treatment responses in a real-world setting [11,13,68–74]. Yet the body of long-term real-world evidence after the first year of treatment is still limited, which is needed to estimate the impact of CFTR modulating therapies on individual CF disease progression.

THE ROLE OF BIOMARKERS IN CLINICAL TRIALS

A biomarker is defined as a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes and biological responses to an exposure or intervention, including therapeutic interventions [75]. In a clinical context, biomarkers can therefore be used to e.g. inform diagnosis, prognosis and to predict treatment effects. In order to be accepted by regulatory authorities as a surrogate outcome in registration trials, validation is required to demonstrate that the biomarker reliably predicts the clinical effectiveness of a medicinal product [75].

CFTR function biomarkers

Several bioassays have been developed that quantify CFTR function, including the SwCl test, nasal potential difference (NPD) and intestinal current measurements (ICM) [61,76]. Even though all three biomarkers have mainly been validated in the context of CF diagnosis, their ability to accurately discriminate between individuals with differential disease progression is limited despite clear relations at a population level [57,61,76–82]. Accumulating data on reliability, validity, responsiveness and feasibility of the SwCl test has suggested its potential as a surrogate endpoint in clinical trials, whereas validity and feasibility of NPD and ICM remain limited in this context [61,76].

Intestinal organoids as a biomarker of CFTR function

Unprecedented advances in stem cell biology have resulted in the development and application of a unique patient-derived intestinal organoid model as an *in vitro* biomarker of CFTR function. Organoids are three-dimensional multicellular structures that comprise tissue features of the parental organ and are usually grown from donor tissue fragments [83]. In CF, human intestinal organoids are generated out of stem cells isolated from rectal biopsies, which is a relatively simple and innocuous procedure that can be performed in all age groups without anesthesia [84]. The forskolin-induced swelling (FIS) assay was developed to quantify CFTR-dependent fluid transport into the intestinal organoid lumen and may provide a more precise and accurate estimation of CFTR function compared to other biomarkers [85,86].

Small proof-of-concept studies have shown that FIS was correlated with biomarkers SwCl and ICM and that clinical disease phenotypes could be stratified based on FIS levels, supporting the association of FIS with CF disease severity [87,88]. Furthermore, responsiveness of FIS to CFTR modulator treatment was also suggested by previous studies which demonstrated an association of FIS with

short-term clinical drug response across groups with different CFTR genotypes [86,89] and in individuals with varying CFTR mutations [90]. In contrast, however, other studies showing a limited or absent FEV1 response were not able to detect an association of FIS and other CFTR biomarkers with short-term clinical response to LUM/IVA in pwCF homozygous for F508del [13], heterozygous for the A455E mutation [14] and to IVA in people with residual function mutations [91].

These pioneering studies created the first context-of-use of FIS as biomarker of CF disease severity and responsiveness to disease-modifying drugs, but further research into the association of FIS with long-term CF disease progression and long-term treatment response in homogeneous and heterogeneous populations with CF is warranted to support validation of the FIS assay and to better understand its potential role as surrogate endpoint.

PROMS IN CLINICAL TRIALS AND CARE

PROMs can be defined as questionnaires that collect information on health status, as experienced and reported directly by the patient [92]. As such, PROMs are generally focused on symptoms, treatment satisfaction, functional status or health-related quality of life [93]. The role of PROMs is becoming increasingly prominent in medical research, as PROMs are considered by regulatory authorities as an essential part of clinical trials for the approval of new drugs or label extension of available drugs [92,94]. When PROMs are meaningful to patients, adequately validated and properly embedded in trials and clinical care, they can support pharmaceutical labeling claims, facilitate treatment reimbursement, assist in shared-decision making and contribute to the transition towards a more value-based and patient-centered healthcare system [92,94–98].

PROMs in CF

In CF, many different generic and disease-specific PROMs are being used in clinical research, although implementation in clinical care remains challenging [99,100]. Since its initial development in the late 1990s in France [101] and subsequent international revisions a few years later [62], the CFQ-R is by far the most commonly used and well-validated disease-specific PROM to measure health-related quality of life in pwCF [99,100]. Although disease-specific PROMs are usually more sensitive and reflective of patient symptoms and functioning than generic PROMs, the relevance of the CFQ-R has diminished over time according to pwCF [100]. This is most likely related to the changing impact of CF in the context of CFTR modulator therapy, which is generally leading to milder clinical symptoms

than before. Furthermore, the respiratory symptom subscale of the CFQ-R is the main focus of most CF-related clinical trials, as it has always been the best validated and most sensitive CFQ-R subdomain [63]. Nevertheless, respiratory symptoms can vary across individuals with differential disease expression and are not completely reflective of an individual's quality of life, stressing the need for a novel, more modern and patient-centered approach to adequately capture the impact of disease, treatment modalities and healthcare on individuals with CF.

AIMS AND THESIS OUTLINE

This thesis aims to assess the utility of long-term real-world data for the development of new biomarkers such as patient-derived intestinal organoids and of novel personalized patient-reported outcome measures, which could ultimately support further development of effective and personalized treatments for all individuals with CF.

Chapter 2 provides an update on the latest developments in diagnostics, treatment and prognosis of CF in the era of targeted therapy, including an overview of potential new treatments that are currently in early stages of development.

To assess the utility of long-term real-world outcomes and the potential role of patient-derived organoids as surrogate endpoint, **chapter 3** evaluates the association between FIS of intestinal organoids and different measures of long-term CF disease progression, which supports validity of the FIS assay as a CFTR-function biomarker of disease severity and progression.

Chapter 4 assesses long-term effectiveness of dual CFTR modulator therapies in pwCF homozygous for the F508del mutation, whereas **chapter 5** describes the predictive value of FIS for long-term clinical response to dual CFTR modulator treatment, in combination with other *in vivo* and *in vitro* biomarkers.

Chapter 6 shows the development and validation of a novel personalized electronic PROM that can be used to assess all aspects of quality of life that matter for individuals with CF, demonstrating its benefits over traditional generic and disease-specific PROMS and its potential application as individualized outcome measure in CF and other rare or heterogeneous diseases.

Finally, **chapter 7** provides a summary of the main findings of the research described in this thesis and a discussion of the most important strengths, limitations,

conclusions and future recommendations that can help to move forward towards a cure for all individuals with CF.

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CHAPTER 2

A new era for people with Cystic Fibrosis

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European Journal of Pediatrics 2021, Sep;180(9):2731-2739

ABSTRACT

Cystic Fibrosis is the most prevalent inherited disease caused by a defect in the *cystic fibrosis transmembrane conductance regulator* (CFTR) gene. The impaired electrolyte homeostasis caused by the mutated or absent protein leads to symptoms in multiple organ systems. However, the pulmonary manifestation with chronic infections and eventually respiratory failure remains the most important threat. Until one decade ago, only symptomatic treatment was available. However, since 2012, different combinations of CFTR modulators are available for people with cystic fibrosis (pwCF) that carry different mutations. The advent of these drugs has impressively changed life expectancy and quality of life in people with cystic fibrosis and raised new challenges regarding long-term complications and tapering of conventional therapies.

Conclusion

In this review, we provide an update on the latest developments around diagnostics, treatment, and prognosis of pwCF.

INTRODUCTION

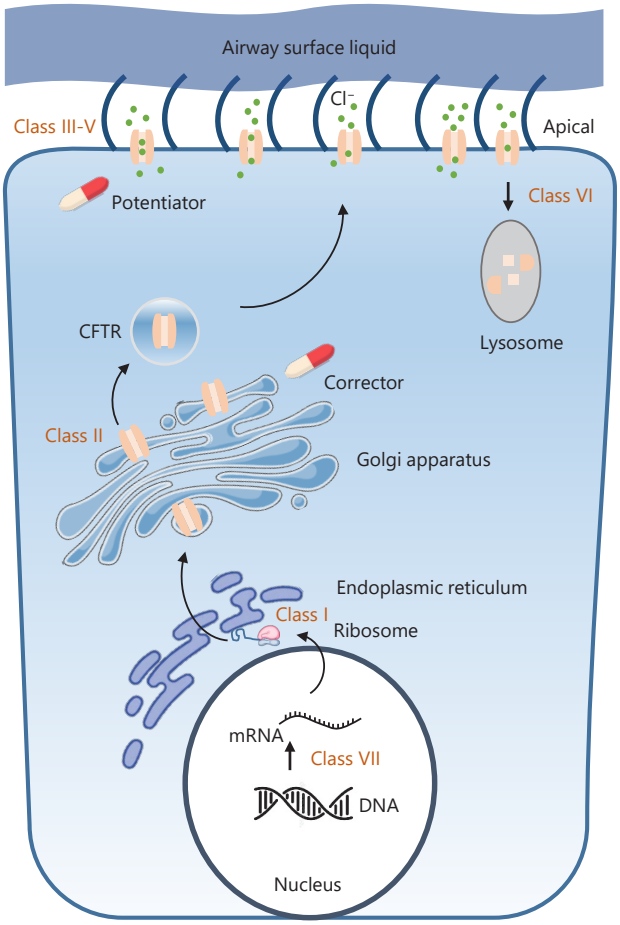
Cystic Fibrosis (CF, OMIM #219,700) is a rare, autosomal recessive, monogenic disease caused by mutations in the *cystic fibrosis transmembrane conductance regulator* (CFTR) gene. The CFTR protein is an essential regulator of many mucosal surfaces' fluid and electrolyte homeostasis [1]. When CFTR is absent or does not function properly, accumulation of viscous mucus in the pulmonary and gastrointestinal tract will occur. This abnormal fluid consistency leads to infections, inflammation, malnutrition, and finally, progressive multi-organ dysfunction.

At this point, over 2000 CFTR mutations have been reported causing a variety of different disease phenotypes (https://cftr2.org). All these mutations result, to some extent, in abnormal chloride and bicarbonate transportation across epithelial cells. Mutations are classified into seven different classes based on functional impairments. Classes I to III are associated with little to no CFTR function and therefore associated with a more severe phenotype. Classes IV to VII have residual CFTR function and tend to be less severe (**figure 1**) [2]. However, there is a wide range of disease severity with a median age of survival approaching 50 years. This disease variation is most clearly related to the type of CFTR mutation, but is also influenced by additional non-CFTR dependent genetic and environmental factors [3].

Globally, there are close to 90,000 people with CF (pwCF) of which 50,000 live in Europe. The prognosis has tremendously changed for the better over the last decade, especially since the first small molecules' market approval treats the underlying defect in CF.

With this review, we provide an update on the latest developments around diagnostics, treatment, and prognosis of pwCF.

Figure 1. Biosynthesis of the CFTR protein and target sites of market approved modulators



CLINICAL PHENOTYPE

CF is often seen as a pulmonary disease; however, the lack of CFTR function affects multiple organ systems. Disease severity and the number of organ systems involved vary from patient to patient. The respiratory manifestations are caused by chronic pulmonary infections, which eventually lead to progressive lung function decline and respiratory failure, which is the leading cause of death for pwCF [4]. Structural lung damage can already be visible on computed tomography images in asymptomatic infants [5]. Besides dense mucous, CFTR dysfunction in bronchial epithelia also leads to increased inflammatory response and impaired immune response, making it prone to acute infections and chronic bacterial

colonization of the lung [6]. The pathogens *Staphylococcus aureus* and *Pseudomonas aeruginosa* are most prevalent. However, when the disease progresses, more unusual pathogens like *Achromobacter xylosoxidans*, *Burkholderia cepacia*, *Strenotrophomonas malthophilia*, and mycobacteria can be cultured of which the latter is more challenging to treat [7]. Along with bacterial infections, pwCF are also more prone to viral infections, which are linked to exacerbations [8]. The last group of pathogens found in the lungs are fungi, particularly *Aspergillus* species. An increased rate of allergic reactions to *Aspergillus* is seen in pwCF. This allergic bronchopulmonary aspergillosis contributes to chronic pulmonary function decline [9]. The upper airways are also frequently affected and often require sinus surgery due to nasal polyposis, mucocele, and sinusitis [10]. Lung function is crucial in monitoring disease progression and is universally measured through spirometry. A disadvantage of this method is that the technique is too difficult for children below the age of 6. Recently, multiple breath washout testing has become important in clinical research and care with the lung clearance index as a primary outcome. This technique is less dependent on patient effort, making it very suitable for the pediatric population [11].

The manifestation of impaired CFTR function in the gastrointestinal tract already starts in utero. In the pancreas, the pancreatic fluid's viscosity is causing obstruction and secondary tissue destruction, resulting in the formation of cysts and fibrosis. Pancreatic exocrine insufficiency is found in 60–80% of pwCF at birth leading to malabsorption and malnutrition when untreated. As pancreatic fibrosis eventually can lead to CF-related diabetes (CFRD), it is recommended to annually screen with an oral glucose tolerance test from the age of 10 [12]. A more rare complication is the occurrence of distal intestinal obstruction syndrome (DIOS) where a complete or incomplete obstruction is seen in the ileocaecum causing nausea, abdominal pain, and hard stools. This should be distinguished from constipation [13]. In the liver, CFTR dysfunction can lead to a broad spectrum of conditions from mild cholestatic disease to cirrhosis, collectively referred to as CF-related liver disease [14].

In addition to CFRD, there are other endocrine manifestations of the disease. Poor growth is not solely due to malnutrition and chronic lung infections; it is suggested that CFTR dysfunction also affects the secretion of growth hormone from the pituitary gland [15,16]. Up to 90% of males have a congenital bilateral absence of the vas deferens (CBAVD) with average sperm production [17]. This can also frequently be seen as an isolated symptom that leads to the diagnosis of CF-related disorder. Women are also less fertile due to impaired CFTR function related changes in the reproductive system [18]. Bone density can also be affected in pwCF, up to 50% of adults have osteopenia which can lead to osteoporosis. The impaired

bone health knows different causes: vitamin D and K deficiency, glucocorticoid therapy, altered sex hormone production, malnutrition, inflammation, and low physical activity rate [19].

DIAGNOSIS

Traditionally, the diagnosis of CF relies on the clinical presentation of the disease. Nowadays, most pwCF are diagnosed after a positive CF newborn screen (NBS). The foundation of the CF-NBS lies in New Zealand, where Crossley et al. made it feasible to analyze dried blood spots for immunoreactive trypsinogen (IRT) [20]. Elevated IRT indicates a significant risk of CF. Ten years after this research, CF-NBS was, in 1980, first implemented in Europe. Nowadays, most European countries have incorporated CF in their NBS programs [21].

The European Cystic Fibrosis Society Patient Registry Annual Data report (2018) shows a European median age at diagnosis of 4 months [4]. Nevertheless, it remains important to know the disease's clinical manifestation to help diagnose patients whose NBS does not pick up. A wide variety of symptoms can lead to the diagnosis (i.e., chronic diarrhea, steatorrhea, malabsorption, nasal polyps). However, the most common presentation is a combination of chronic or recurrent respiratory tract infections and malabsorption, prompting the diagnosis of CF [22]. Another important clinical manifestation seen in 20% of pwCF is meconium ileus. Due to the high correlation between meconium ileus and CF, it is essential to be aware that NBS can be falsely negative in children with meconium ileus. Therefore, it is still recommended to perform additional tests (sweat and/or genetic test) in clinical symptoms despite a negative NBS [23].

Once the diagnosis is suspected, either through a positive NBS or clinical manifestations, referral to a specialized CF center and additional testing is needed. The first step in diagnostics is to measure (dys)function of the CFTR channel, followed by genetic testing. The most reliable and widely used test is the measurement of chloride concentration in sweat (SCC) sometimes complemented with electrophysiological tests. In Europe, three different diagnostic categories are recognized and distinguished by different SCC levels: (1) (typical) CF, (2) atypical/non-classic CF, and (3) CFTR-related disorder (CFTR-RD) [22]. The first category is clearly described as the combination of CF specific symptoms and a SCC above 60 mmol/L on two occasions. The second category is not recognized in the USA. However, it is used for a group with borderline SCC levels (30–60 mmol/L) in combinations with CF specific symptoms and CFTR dysfunction proven by two CF

causing CFTR mutations or an abnormal function test. CFTR-RD is diagnosed when a patient shows disseminated bronchiectasis, recurrent pancreatitis, or congenital bilateral absence of the vas deferens together with only one CF causing CFTR mutation or borderline SCC levels [24].

When a newborn, after a positive NBS, does not fully meet the diagnostic criteria for CF and does not show any clinical signs, the term cystic fibrosis transmembrane conductance regulator-related metabolic syndrome/cystic fibrosis screen positive, inconclusive diagnosis (CRMS/CFSPID) is used. The first part, CRMS, knows its origin in the USA, and CFSPID was used in other countries; the terms were combined in 2016 to ease the collection of data and improve patient care [25]. In 2020, an updated guidance was published on the clinical management of these children. Most of these children will never develop any clinical symptoms and remain healthy, an unknown part however will eventually be diagnosed with CF or CFTR-RD. At this point, it cannot be predicted who will develop CF and early recognition is very important. It is therefore recommended to thoroughly examine these newborns and proceed with a yearly check up until, at least, the age of 6 years. The check up at year 6 has been enhanced with the advice to perform a pulmonary function test and chest imaging [26].

TREATMENT

The multi-organ involvement in CF makes it a complex disease to treat. Therefore, pwCF should always receive care in a specialized CF center where care is provided by a multi-disciplinary team consisting of at least a specialist physician, nurse specialist, physiotherapist, dietician, psychologist, and a social worker [27]. The treatment regimen has changed drastically since Dorothy Andersen first described the disease in 1938, in a pre-antibiotic era [28]. Until a decade ago, all therapies were solely based on the treatment of symptoms due to loss of CFTR function. There are airway clearance techniques and nebulized drugs for mucus obstruction, oral and inhaled antibiotics for infections, and pancreas enzymes for malabsorption. They have all led to substantial improvement in life expectancy and quality of life.
Nonetheless, ever since the discovery of the CFTR gene in 1989, researchers have been determined to find targeted therapy to improve the function of mutant CFTR proteins. This breakthrough had led to the development of CFTR modulators that made their entry into the market almost 10 years ago. These new targeted therapies are causing a tremendous shift in the care for pwCF. Trials are currently being organized to see if and which part of the symptomatic treatment can be ceased after starting with modulator therapy [29].

Symptomatic treatment

Despite the exciting emergence of CFTR modulator therapy, symptomatic therapy still plays an important role in the treatment of pwCF. Not all pwCF will have access to these drugs due to age or genotype. We know that early introduction of therapy targeting the downstream effects of CF is important for disease severity later in life. For instance, recovery of lower birth weight at the age of two is correlated with better pulmonary outcome at 12 years [30]. The keystones in daily CF treatment are pancreatic enzyme replacement therapy, airway clearance therapies, and antimicrobial treatments.

Pancreas enzymes (lipase, amylase, and protease) need to be taken with every meal in case of pancreas insufficiency. All pwCF take vitamin A, D, and E supplements, and on indication (i.e., severe malnutrition and liver failure), vitamin K is added.

One of the main problems in the current treatment is the high prevalence of pulmonary infections with resistant pathogenic organisms [31]. The development of evidence-based guidelines for antibiotic treatment has become more critical in relation to antibiotic resistance and in addition to the development of new therapies. Currently, different studies are being performed with pharmaceutical agents that can disrupt the biofilms, mainly seen in *Pseudomonas* infections, to enhance antibiotic penetrance [32].

Mucociliary clearance therapy is important to increase the viscosity of mucus in the lungs. At this moment, nebulizing hypertonic saline and mannitol form the basis to hydrate the airways. Recombinant human deoxyribonuclease (rhDNase), however, remains the most important pharmaceutical intervention in lowering the viscosity. These therapies are all supplementary to physiotherapy and exercise.

(Highly effective) modulator therapy

CFTR modulating drugs (CFTR modulators) are the first drugs that succeed to treat the underlying genetic defect of cystic fibrosis and thereby to change the lives of pwCF. They have the unique potential to prevent disease expression and limit disease progression. At this moment, four different combinations of modulators are available, all small molecules.

Ivacaftor is the first modulator that got market approval by the EMA in 2012, specifically for patients with a G551D gating mutation (class III) [33]. Later the label has been extended to 38 other mutations, which covers ~ 4% of pwCF worldwide [34]. Ivacaftor is a so-called potentiator, and it increases the amount of time that the CFTR channel is open, improving the chloride transport through the CFTR channel.

Randomized clinical trials showed a clear positive effect on lung function, weight gain, and quality of life in different age groups [33,35]. The average increase of percent predicted forced expiratory volume in 1 s ($ppFEV_1$) was about 10%. Most clinical trials base their outcome on short-term data, measured weeks after the start of treatment. However, there is evidence that even patients that do not show any short-term response could benefit from ivacaftor. A study has been performed that compared the outcomes of short-term responders and non-responders over 2 years in relation to the pre-treatment baseline. This showed strong evidence that ivacaftor is also beneficial when no short-term improvements in $ppFEV_1$ and/or BMI is measured. The strongest outcome was a 50% reduction in pulmonary exacerbations in both pre-and-post ivacaftor treatment [36]. Long-term data in a G551D population shows a sustainable effect on multiple outcome levels, including lung function, after 5.5 years of ivacaftor [37]. There is also evidence that treatment with ivacaftor has a positive effect on both insulin secretion in people with abnormal glucose tolerance and hepatic steatosis in people with CF-related liver disease [38,39]. In September 2020, the European Medicines Agency (EMA) lowered the minimum age to 4 months. A pivotal study in a ferret model showed that in utero treatment could partly prevent disease development until discontinuation of the treatment [40].

Two double therapies lumacaftor/ivacaftor (LUM/IVA) and tezacaftor/ivacaftor (TEZ/IVA) got market authorization in 2015 and 2018, respectively. The two additions to ivacaftor are both CFTR modulators that function as a corrector. They stabilize the CFTR protein and rescue intracellular trafficking to the cell surface. The corrected CFTR that reaches the cell surface is then potentiated by ivacaftor to improve function further. Clinical effects of LUM/IVA are modest with a $ppFEV_1$ increase of 2.6% in a F508del homozygous group and not significant in people with only one F508del mutation [41]. Although LUM/IVA and TEZ/IVA have a comparable working mechanism, TEZ/IVA shows a more favorable outcome in terms of pulmonary adverse events and drug interaction profile. People that had to quit treatment with LUM/IVA due to treatment-related respiratory symptoms tolerated the switch to TEZ/IVA very well [42]. The average improvement in $ppFEV_1$ in homozygous F508del patients is 3–4% [43,44]. While LUM/IVA is only registered for F508del homozygous pwCF, TEZ/IVA is also approved for F508del with an additional residual function mutation, from 6 years and older.

In June 2020, EMA approved the triple combination elexacaftor/tezacaftor/ivacaftor. Here the additive compound elexacaftor is, like tezacaftor, a CFTR corrector but putatively binds to a different protein site than tezacaftor. A recent *in vitro* study showed that elexacaftor also exhibits the activity of a potentiator [45]. The triple combination has been the most potent combination so far and shows

spectacular improvement on all measured outcomes including an increase of 14.3% $ppFEV_1$ [46]. A phase 3 trial found a 10% higher increase of $ppFEV_1$ in the triple group compared to the TEZ/IVA group [47]. Increase in pulmonary function and weight remains stable over time, at least for 48 weeks [48]. The phase 2/3 clinical trials with CFTR modulators use inclusion criteria that exclude subjects with either low or high pulmonary function, $ppFEV_1$ < 40% or higher than 90%, respectively. A large prospective observational study showed that pwCF with a $ppFEV_1$ below 40% that use the triple therapy as part of a "temporary use program" also show great response with a mean increase of $ppFEV_1$ of 15.1% [49]. Although responses on group level are impressive, there is still a wide range in response with $ppFEV_1$ change ranging from −2.5 to > 20% [47]. In March 2021, EMA extended approval, in line with FDA, for pwCF that carry at least one F508del mutation. The FDA extended their label in December 2020 with an additional list of 177 rare mutations and lowered the age from when it can be prescribed in June 2021 to 6 years.

Unfortunately, not all pwCF can benefit from these highly effective modulator drugs because their (rare) mutation is not listed for reimbursement. Currently, there are multiple pharmaceutical companies that have modulator therapies in their pipeline. Additionally, a European project called "Human Individualized Treatment for CF" (HIT-CF) is ongoing in 16 different countries. The goal of the project is to get modulator drugs to pwCF that carry (ultra)-rare mutations by predicting clinical drug response by testing the mini-guts (organoids) of these patients *in vitro* [50,51]. Overall, the advent of these CFTR modulators will be life-changing for up to 90% of pwCF.

Future therapeutics to correct CFTR

On top of the different pharmaceutical companies that are developing competing CFTR modulators, there are also CFTR modulators with different mechanisms of action that have entered the clinical pipeline (**table 1**). Currently, a phase 2 trial is conducted with ELX-02, a read-through compound, designed for pwCF that carry nonsense mutations. Preclinical data show encouraging improvements in CFTR function measured in organoids from pwCF carrying the most prevalent nonsense mutation G542X [52]. Another promising development lies in the field of gene therapy. At this moment, the first trial is being conducted with mRNA therapy where normal CFTR-encoded mRNA is delivered to the lungs by a nebulizing device [53]. This therapy would work for all pwCF regardless of their individual mutation. The downside of this type of therapy is that, for now, the technique can only be applied locally in the lungs due to the instability of mRNA. This means that these people will still suffer from CF-related morbidities such as CFRD and malabsorption due to pancreatic insufficiency. Therefore, it would be exciting to look into combination therapies with different actions to maximize the restoration of CFTR function in all affected organs.

Table 1. Overview of current (pre)clinical treatments to restore CFTR function

Compound	Developmental stage	Mode of action
ABBV-2222	clinical; phase 2	corrector
ABBV-3067	clinical; phase 2	potentiator
ELX-02	clinical; phase 2	read-through
PTI-801	clinical; phase 2	corrector
PTI-808	clinical; phase 2	potentiator
PTI-428	clinical; phase 2	amplifier
ABBV-3067	clinical; phase 1	potentiator
MRT5005	clinical; phase 1	mRNA (inhaled)
RPL554	clinical; phase 1	phosphodiesterase 3/4 inhibitor
VX-121	clinical; phase 1	corrector
VX-561	clinical; phase 1	potentiator
ARCT-032	pre-clinical	mRNA (inhaled)
ARCT-032	pre-clinical	mRNA (inhaled)
SPIRO-2101	pre-clinical	gene therapy (inhaled)
SPIRO-2102	pre-clinical	gene therapy (inhaled)
4D-710	pre-clinical	gene therapy (inhaled)

With the emergence of highly effective therapies, it is essential to evaluate the option to reduce the daily treatment burden of pwCF. A recent study showed that 81% of current CFTR modulator users did not stop any chronic treatment, while supporting both the CF community as CF physicians to assess this more thoroughly. Airway clearance techniques and inhaled antibiotics are considered the most significant contributors to treatment burden [54]. In 2020, a randomized clinical trial (SIMPLIFY, NCT04378153) started to see if hypertonic saline and rhDNase can safely be withdrawn from the daily treatment regimen [29]. It is also crucial to answers this question for other domains such as antibiotic use and dietary advice.

PROGNOSIS

The enormous change in therapeutic development and treatment regimen has changed the life expectancy of pwCF tremendously. Cystic Fibrosis used to be a childhood disease, but the latest registry data shows that 51.2% of all pwCF in Europe are adults [4]. Until the 1980s, life expectancy was around 18 years old. This was also the time that the CF-NBS was introduced, the mucolytic agent rhDNase, and different antibiotics for inhalation became available [55]. Now 40 years later, life expectancy has more than doubled and reaches 50 years in high-income countries [3]. For low- and middle-income countries like Brazil, South Africa, and India, these numbers are significantly lower. These countries have other sizeable public health challenges to overcome like tuberculosis, human immunodeficiency virus,

and community-acquired pneumonia. In these countries, CF is not a priority to the government and symptoms may be attributed to other diseases than CF [56]. Putting the differences between countries aside, there are also inequities within countries due to socioeconomic status differences. Examples of factors contributing to this inequity are second-hand smoking, air pollution, national status, and psychological functioning [57].

The overall increase in life expectancy comes with new challenges and asks for new strategies in preventing long-term complications. For instance, it is known that there is a relatively high prevalence of anxiety and depression in pwCF and that starting modulating drugs could potentially worsen symptoms while general health improves [58]. Another example is the need to assess the impact of modulator use in pregnancy. Survey studies so far imply that modulators can safely be used but more data is needed [59]. Also, dietary guidelines need to be adjusted. Significant weight gain is seen in pwCF on modulating drug which should be aware of the problem of obesity. Increased risk for intestinal cancer and cardiovascular complications will ask to develop preventive screenings programs and early interventions when pwCF grow older in the near future [60–62].

CONCLUSION

Life expectancy for pwCF is impressively improving due to the treatment with CFTR modulators and high standard of care in CF centers. For a subpopulation of pwCF who are not eligible for CFTR modulator therapy, there is still a desperate need for new therapies. Since the early start of treatment can prevent many of the disease manifestations, it remains crucial to be alert on the diagnosis CF. Altogether, the 2020s will be a new era for pwCF with effective therapies on the market and many more on the way.

Author contributions

Writing - initial draft preparation: Marlou C. Bierlaagh; Writing - review and editing: Danya Muilwijk, Jeffrey M. Beekman and Cornelis K. van der Ent.

Conflicts of interest

CKvdE and JMB report grants from Eloxx, Galapagos NV, Gilead, GSK, Nutricia, ProQR, Proteostasis, Teva, and Vertex Pharmaceuticals Incorporated, and a patent (10006904) with royalties paid.

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CHAPTER 3

Forskolin-induced organoid swelling is associated with long-term Cystic Fibrosis disease progression

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European Respiratory Journal 2022, Aug 18;60(2):2100508

ABSTRACT

Rationale

Cystic fibrosis (CF) is a monogenic life-shortening disease associated with highly variable individual disease progression which is difficult to predict. Here we assessed the association of forskolin-induced swelling (FIS) of patient-derived organoids with long-term CF disease progression in multiple organs and compared FIS with the golden standard biomarker sweat chloride concentration (SCC).

Methods

We retrieved 9-year longitudinal clinical data from the Dutch CF Registry of 173 people with mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene. Individual CFTR function was defined by FIS, measured as the relative size increase of intestinal organoids after stimulation with 0.8 μM forskolin, quantified as area under the curve (AUC). We used linear mixed-effect models and multivariable logistic regression to estimate the association of FIS with long-term forced expiratory volume in 1 s % predicted (FEV_1pp) decline and development of pancreatic insufficiency, CF-related liver disease and diabetes. Within these models, FIS was compared with SCC.

Results

FIS was strongly associated with longitudinal changes of lung function, with an estimated difference in annual FEV_1pp decline of 0.32% (95% CI 0.11–0.54%; p=0.004) per 1000-point change in AUC. Moreover, increasing FIS levels were associated with lower odds of developing pancreatic insufficiency (adjusted OR 0.18, 95% CI 0.07–0.46; p<0.001), CF-related liver disease (adjusted OR 0.18, 95% CI 0.06–0.54; p=0.002) and diabetes (adjusted OR 0.34, 95% CI 0.12–0.97; p=0.044). These associations were absent for SCC.

Conclusion

This study exemplifies the prognostic value of a patient-derived organoid-based biomarker within a clinical setting, which is especially important for people carrying rare *CFTR* mutations with unclear clinical consequences.

INTRODUCTION

Clinical disease expression in people with cystic fibrosis (CF) is variable and results from a combination of genetic, environmental and stochastic factors that are unique for each individual. CF is a recessive, monogenic disease caused by mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene [1]. More than 2000 *CFTR* variants which differentially affect CFTR function and clinical phenotype have been identified (https://cftr2.org). The more common mutations have been categorised into distinct classes according to the mechanism by which CFTR function is disrupted [2]. To better understand how CFTR function contributes to disease expression, biomarkers such as sweat chloride concentration (SCC), intestinal current measurements (ICM) and nasal potential difference (NPD) are used to estimate individual CFTR function. These biomarkers have mostly been validated in the context of CF diagnosis, but their ability to accurately discriminate between people with CF with differential disease progression is limited despite clear relationships at population level [3–9]. Forskolin-induced swelling (FIS) of patient-derived intestinal organoids is an *in vitro* biomarker that quantifies CFTR-dependent fluid transport into the organoid lumen [10,11] and may provide a more precise and accurate estimation of CFTR function compared to other biomarkers. Small proof-of-concept studies showed that FIS correlates with SCC and ICM and that clinical disease phenotypes could be stratified based on FIS level [12,13]. We hypothesised that individual CFTR function measured by FIS is associated with long-term disease progression defined by rate of forced expiratory volume in 1 s % predicted (FEV_1pp) decline and development of comorbidities such as pancreatic insufficiency, CF-related liver disease and CF-related diabetes. Such an association supports a potential role for FIS as biomarker for long-term disease progression, which is especially relevant to people with rare, uncharacterised *CFTR* genotypes or *CFTR* genotypes with varying clinical consequences.

METHODS

Study design and population

A longitudinal cohort study was conducted in Dutch people carrying mutations in the *CFTR* gene who are included in the Dutch Cystic Fibrosis Registry (DCFR). For all participants, intestinal organoids were generated before January 2020 and written informed consent was obtained to use their intestinal organoids and clinical data for the present study. This study was approved by the institutional review board of the University Medical Center Utrecht (Utrecht, the Netherlands).

Study parameters

The primary outcome variable was defined as long-term lung function decline, expressed as FEV_1pp, calculated according to Global Lung Function Initiative guidelines [14]. Secondary outcome variables were occurrence of pancreatic insufficiency, defined by faecal elastase <200 $\mu g \cdot g^{-1}$; CF-related liver disease, defined by hepatic steatosis or cirrhosis confirmed by imaging; and occurrence of insulin-dependent CF- related diabetes, defined by daily insulin treatment.

The primary explanatory variable of interest was FIS, defined by the relative size increase of intestinal organoids after 1 h stimulation with 0.8 μM forskolin, quantified as area under the curve (AUC). Previous studies showed that discrimination between individual FIS responses was most optimal and correlated best with other *in vitro* and *in vivo* CFTR biomarkers when FIS was performed with 0.8 μM forskolin [11,12]. Other explanatory variables included were age (in years) at time of each lung function measurement; treatment status at time of each lung function measurement, categorised as no CFTR modulator treatment, treatment with ivacaftor or with lumacaftor/ivacaftor; sex; SCC in $mmol \cdot L^{-1}$; and genotype, categorised as class I–V or unclassified, defined by genotype class of the mildest of both mutations according to the available literature (**supplementary tables S1** and **S2**). Additionally, genotypes were categorised in groups according to the combination of the following mutation types: insertion/deletion, nonsense, missense, splice and unknown.

Study procedures

Organoid measurements

The generation of intestinal organoids from biopsies and subsequent fluid secretion assays (FIS-assays) were performed according to a previously described protocol [15]. Rectal biopsies were collected at one time point during the 9-year study period. The specific time point of rectal biopsy collection varied per study participant, but was always prior to the start of modulator therapy. FIS-assays were performed between 2014 and 2020 by analysts who were blinded for genotype and clinical data. All FIS-assay experiments were conducted in duplicate and for the majority of the donors at multiple culturing time points with a maximum of seven consecutive culture time points (n=7).

Clinical data collection

Data on clinical study parameters were retrieved from the DCFR, independent of FIS-assay results. Annual best FEV_1pp values between 2010 and 2018 were used to estimate lung function decline. Treatment status at the time of each lung function measurement was calculated based on start and stop dates of CFTR modulators as

registered in the DCFR. For SCC, pancreatic insufficiency, CF-related liver disease and CF- related diabetes, we only collected the most recent value registered before 2019 (or before CFTR modulator treatment initiation, if applicable), as repeated measurements were unavailable or inconsistently collected. For SCC, pancreatic insufficiency, CF-related liver disease and CF-related diabetes, data were missing in 59 (34.1%), 63 (36.4%), five (2.9%) and three (1.7%) participants, respectively. SCC values were mostly missing for older participants, which may have been performed years before the start of the registry in 2010 and were not archived within the local CF centres.

Statistical analysis

The association between age and long-term lung function decline was analysed using a linear mixed-effects model. FEV_1pp was specified as outcome variable in the model, with FIS, SCC, genotype class (reference category: unclassified), sex (reference category: male), age, CFTR modulator treatment (reference category: none) and FIS×age as fixed effects, where the interaction term FIS×age reflected the difference in annual FEV_1pp decline by FIS level. The model included a random intercept and random slope for age per subject, assuming a first-order auto-regressive (cAR1) correlation structure. Conditional R^2 was calculated to assess overall model performance and marginal R^2 to estimate the relative contribution of the fixed effects.

To account for selection bias towards a milder phenotype in participants surviving to an older age, a subgroup analysis was conducted including measurements performed between 4 and 25 years of age, in which the relationship between age and FEV_1pp decline can reasonably be assumed to be linear in this dataset (**figure 2a**).

Sensitivity analyses were performed using genotype group, defined by the combination of mutation types, *e.g.* insertion/deletion, nonsense, missense, splice, unknown. Genotype group was used instead of genotype class, to assess whether the association of FIS with FEV_1pp decline was influenced by categorisation of genotype. To obtain reliable effect estimates and standard errors for genotype group, groups with less than five participants were excluded from this part of the analysis.

To compare the association of long-term FEV_1pp decline with FIS *versus* SCC, four models were built which all included FIS, SCC, genotype class, sex, age and treatment as fixed effects. A baseline model was built without any interaction term, and the other three models were built with the addition of either the interaction term FIS×age, SCC×age or both FIS×age and SCC×age in the model. Performance of

these models was compared using the likelihood ratio test.

Multilevel multiple imputation based on the method of chained equations [16] was used to handle missing SCC data in the linear mixed-effects models. All analyses were performed on 20 imputed datasets (m=20, iterations=20) with pooling of the results.

Secondary outcomes were analysed using multivariable logistic regression, with FIS, SCC, sex and age at the last study measurement as explanatory variables. Given the low proportion of outcome events within some of the genotype classes as well as within genotype groups (defined by the combination of the mutation types on both alleles), genotype could not be included in these analyses. In addition, CFTR modulator usage was not included, as we only collected most recent values of pancreatic insufficiency, CF- related liver disease and CF-related diabetes before modulator initiation. Nagelkerke's R^2 was calculated to assess model performance. Single-level multiple imputation [16] was used to handle missing data of SCC, pancreatic insufficiency and CF-related diabetes in the logistic regression models. The analyses were performed on 20 imputed datasets (m=20, iterations=20) with pooling of the results.

Significance levels were set at 0.05. All statistical analyses were performed with R version 4.1.1 using packages mice, micemd, nlme and lme4 in combination with the performance package.

RESULTS

Participant characteristics

In total, 173 participants carrying different *CFTR* genotypes provided written informed consent to collect intestinal organoid data and retrieve their clinical data from the DCFR. Participant characteristics are summarised in **table 1**. Three participants were excluded from the analysis because clinical data were not available. No data were excluded based on organoid measurements. Classification per mutation, individual genotypes with corresponding mutation classification and mutation group are listed in **supplementary tables S1** and **S2,** respectively.

Table 1. Participant characteristics (n=173)

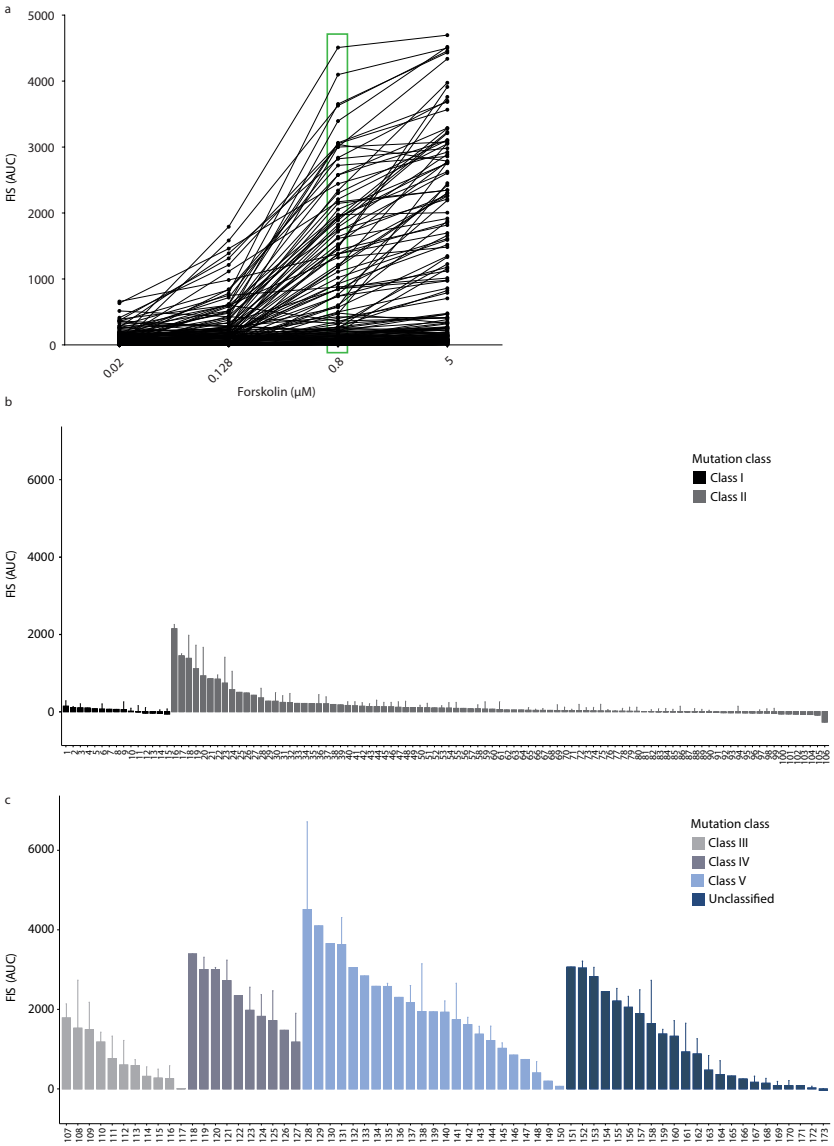
Age	19.5 (9.5 – 30.5)
Sex	
Male	87 (50.3)
Female	86 (49.7)
Mutation class#	
Class I	15 (8.7)
Class II	91 (52.5)
Class III	11 (6.4)
Class IV	10 (5.8)
Class V	23 (13.3)
Unclassified	23 (13.3)
CFTR modulator usage	
Ivacaftor	16 (9.2)
Lumacaftor/ivacaftor	8 (4.6)
FIS¶	141.3 (30.3 – 1176.3)
SCC (mmol·L^{-1})	92.6 (25.9)
Missing values	59 (34.1)
FEV_1pp	75.9 (23.2)
Pancreatic function	
Insufficient (fecal elastase <200 µg/g)	75 (43.4)
Sufficient (fecal elastase ≥200 µg/g)	35 (20.2)
Missing values	63 (36.4)
CF-related liver disease	44 (25.4)
Missing values	5 (2.9)
CF-related diabetes	25 (14.5)
Missing values	3 (1.7)

Data are presented as n, median (interquartile range), n (%) or mean±sd. CFTR: cystic fibrosis transmembrane conductance regulator; FIS: forskolin-induced swelling; SCC: sweat chloride concentration; FEV_1pp: forced expiratory volume in 1 s, % predicted; CF: cystic fibrosis. #: genotype class of the mildest of both mutations; ¶: defined as the relative size increase of intestinal organoids (area under the curve) after 1 h stimulation with 0.8 µmol·L–1 forskolin.

Individual FIS responses

Individual FIS responses after 1 h of stimulation with different forskolin concentrations are shown for all participants in **figure 1a**. Between-subject variability was most apparent at 0.8 µM and 5.0 µM forskolin, but no evident clustering was observed. Consistent with prior studies investigating relations between FIS and CF disease or biomarkers [11,12,17], our analyses were performed with FIS levels upon 0.8 µM forskolin stimulation. FIS data at 0.8 µM forskolin was skewed and highly variable among participants (median, interquartile range (IQR) AUC 141.3, 30.3–1176.3; range –268.0–4508.8; **figure 1a** and **supplementary figure S1a**) as well as within genotype classes (**figure 1b,c**) and between genotype groups, defined by the combination of the two mutation types (**supplementary figure S1b**). As expected, most organoid cultures that showed residual CFTR function (AUC >750) expressed genotypes belonging to classes III–V (**figure 1c**). Surprisingly, seven organoid cultures expressing genotypes categorised as class II mutation, a class for which no residual organoid swelling upon stimulation with 0.8 µM for 1 h has been reported previously [11–13], exhibited moderate to high organoid swelling (**figure 1b**).

Figure 1. Forskolin-induced swelling (FIS) levels of organoids derived from the 173 study participants



a) FIS levels, defined by relative size increase of intestinal organoids after 1 h stimulation with four ascending forskolin concentrations, quantified as area under the curve (AUC). Each line represents swelling of organoids of individual study participants. Each data point represents mean AUC of both technical (n=2) and biological replicates (ranging from n=1 to n=7). b and c) Waterfall plots of FIS responses at 0.8 μM forskolin (highlighted by the green box in a)) of all study participants grouped based on b) mutation class I or II or c) mutation classes III–V or unclassified. Genotypes are categorised into one mutation class based on the mildest mutation class of the two alleles. Bars represent mean+SD of all replicates. Corresponding genotypes for the numbered participants are specified in **supplementary table S2**.

Association of long-term FEV_1pp decline and FIS

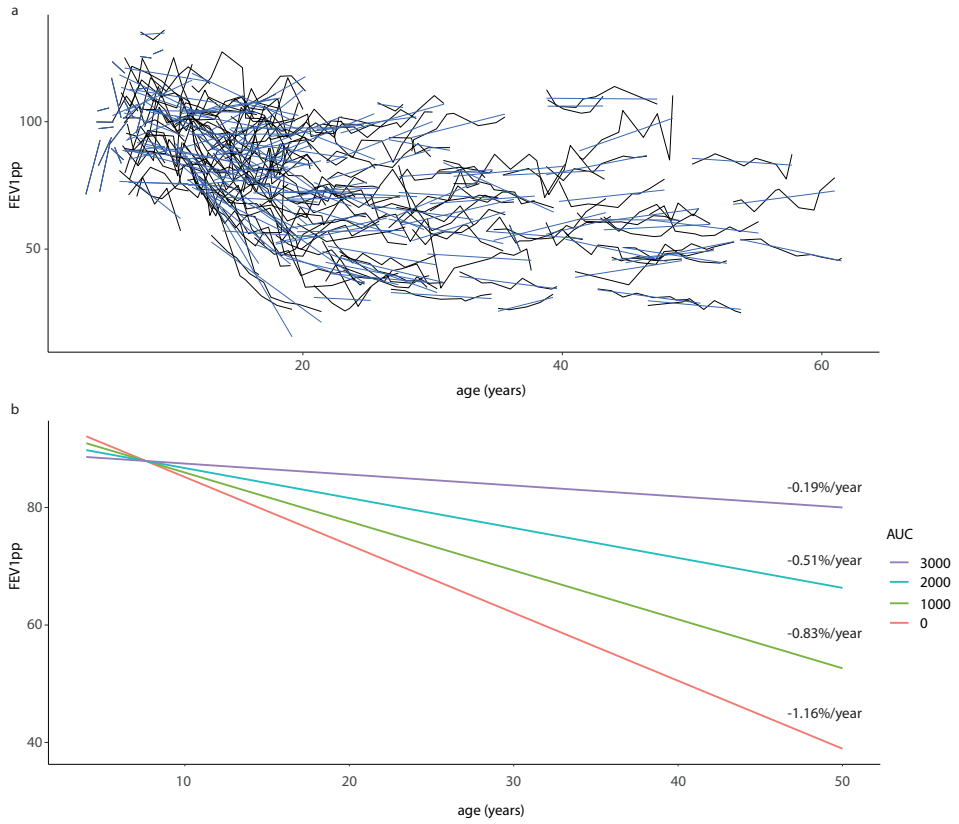
1054 observations of 149 participants with available FEV_1pp measurements (**figure 2a**) were included in the analysis to assess the association of FIS with long-term FEV_1pp decline. Linear mixed-model analysis showed that average FEV_1pp decline per year of age varied with FIS level, adjusted for sex, genotype class, CFTR modulator usage and SCC **(table 2**). To illustrate this association of FEV_1pp decline by age with FIS, **figure 2b** shows that average annual FEV_1pp decline was −1.16% (95% CI −1.43%– −0.88%; p<0.001) per year of age for participants with a FIS level of 0. Per 1000-point increase in AUC, FEV_1pp decline was 0.32% (95% CI 0.11–0.54%; p=0.004) per year of age lower, leading to a very mild estimated FEV_1pp decline of only −0.19% per year for participants with an AUC of 3000. Model performance was excellent based on a pooled conditional R^2 of 0.979 (pooled marginal R^2=0.179).

Table 2. Association of forskolin-induced swelling (FIS)# with forced expiratory volume in 1 s % predicted (FEV_1pp) decline

	Coefficient (95% CI)	P-value
Age	-1.16 (-1.43 – -0.88)	<0.001*
FIS	-2.47 (-8.92 – 3.99)	0.454
FIS*age¶	0.32 (0.11 – 0.54)	0.004*
Treatment		
none	Reference category	
ivacaftor	7.99 (4.58 – 11.40)	<0.001*
lumacaftor/ivacaftor	-3.83 (-8.28 – -0.62)	0.092
Sex		
male	Reference category	
female	-0.96 (-7.00 – 5.08)	0.754
Genotype class+		
unclassified	Reference category	
class I	0.18 (-13.92 – 14.27)	0.980
class II	5.13 (-5.76 – 16.01)	0.356
class III	10.25 (-3.79 – 24.28)	0.152
class IV	11.01 (-5.36 – 27.38)	0.187
class V	-2.31 (-16.95 – 12.33)	0.757
SCC	-0.09 (-0.25 – 0.06)	0.239

Regression coefficients of linear mixed-effects model for FEV_1pp. n=149, n=1054 observations. SCC: sweat chloride concentration. #: defined as the relative size increase of intestinal organoids (area under the curve (AUC)) after 1 h stimulation with 0.8 μM·L−1 forskolin, coefficient scaled 1:1000 AUC; ¶: indicates the difference in annual FEV_1pp decline per 1000 AUC change in FIS level; +: cystic fibrosis transmembrane conductance regulator (CFTR) protein function class of the mildest of both CFTR mutations. Pooled conditional R2=0.979, marginal R2=0.179. *: p<0.05.

Figure 2. Association of forskolin-induced swelling (FIS) with long-term forced expiratory volume in 1 s % predicted (FEV_1pp) decline



a) Individual FEV_1pp trajectories of study participants over time in years. Black lines represent individual observed FEV_1pp trajectories, whereas the blue lines represent estimated average annual FEV_1pp slope per individual. b) Predicted FEV_1pp decline based on linear mixed-effects model coefficients in **table 2**, illustrating the association between different levels of residual cystic fibrosis transmembrane conductance regulator (CFTR) function and long-term FEV_1pp decline. Analysis was performed with FIS as a continuous variable, yet for illustrative purposes predicted FEV_1pp decline is plotted by steps of 1000-point change in area under the curve (AUC). Average predicted annual FEV_1pp decline per 1000 AUC is specified on the right. The lower limit of the x-axis was set at 4 years, because the feasibility and generalisability of FEV_1pp measurements is limited for younger children. Pooled conditional R^2=0.977, marginal R^2=0.179.

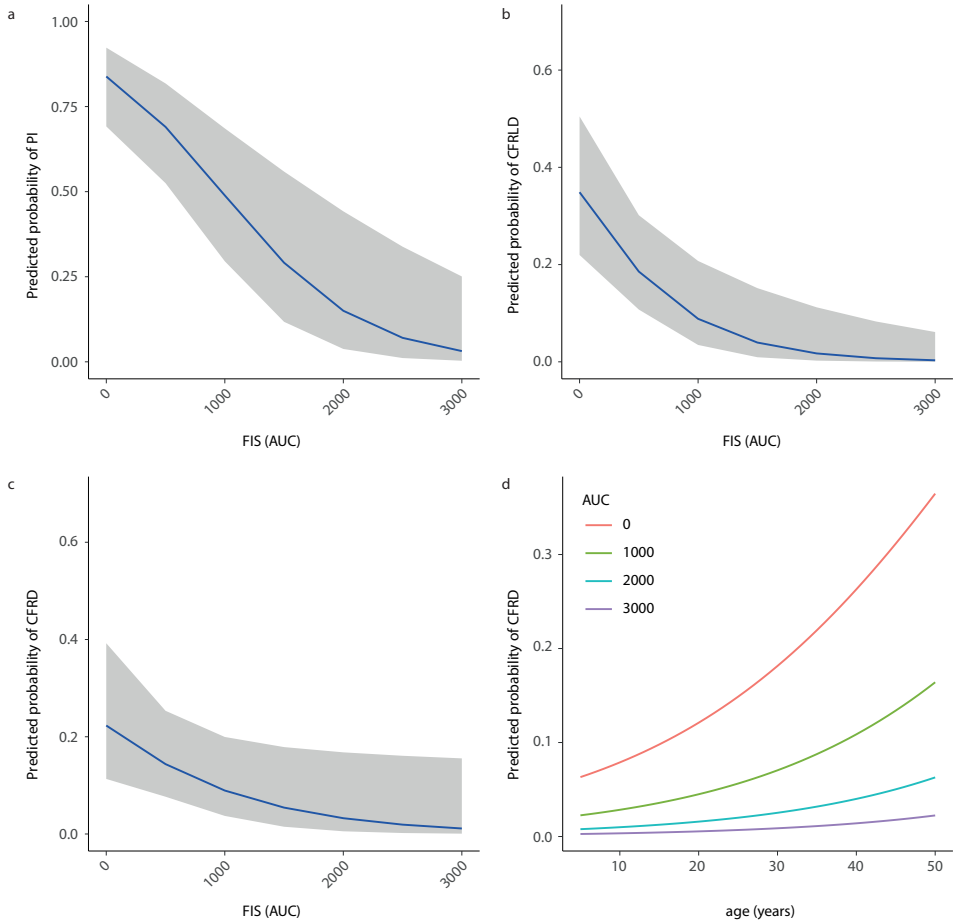
The validity of these results was verified by assessing the potential impact of selection bias and confounding with separate subgroup and sensitivity analyses. A subgroup analysis in participants aged between 4 and 25 years showed a slightly higher average annual FEV_1pp decline compared to the complete population (−1.57% per year, 95% CI −2.03– −1.10%; p<0.001). Similar to the analysis in the complete cohort, FEV_1pp decline varied by FIS level with 0.49% (95% CI 0.03–0.96%; p=0.039; **supplementary table S3** and **supplementary figure S2)** per 1000-point change in AUC, suggesting a negligible impact of selection bias due to the inclusion of people with *CFTR* mutations who have a milder phenotype and survive to an older age. Since at least one *CFTR* mutation was unclassified in 13.3% of participants (**figure 1c**, **table 1** and **supplementary tables S1** and **S2**), a sensitivity analysis was performed in which we refitted both models with genotype group instead of genotype class, to assess whether the association of FIS with FEV_1pp decline was influenced by categorisation of genotype. The association of FIS with FEV_1pp decline in these models was still statistically significant, comparable to the models categorising genotype by mutation class (**supplementary table S4**).

In addition, we compared the association of FIS with FEV_1pp decline *versus* SCC with FEV_1pp decline in similar linear mixed models. SCC alone was not significantly associated with FEV_1pp decline (p=0.121; **supplementary table S5**). An association with SCC was also absent (p=0.995; **supplementary table S6)** when combined with FIS in the model, suggesting a stronger association of FIS with FEV_1pp decline compared to SCC. However, these results should be interpreted with caution due to the proportion of missing SCC data and the use of multiple imputation.

Association of CF-related comorbidities and FIS

To investigate the association of FIS with the occurrence of other CF-related comorbidities, we performed multivariable logistic regression with pancreatic insufficiency, CF-related liver disease and CF-related diabetes, adjusted for age, sex and SCC. We found a significant association of FIS with the occurrence of pancreatic insufficiency (adjusted OR 0.18, 95% CI 0.07–0.46; p<0.001, Nagelkerke's R^2=0.496), CF-related liver disease (adjusted OR 0.18, 95% CI 0.06–0.54; p=0.002, Nagelkerke's R^2=0.222) and CF-related diabetes (adjusted OR 0.34, 95% CI 0.12–0.97; p=0.044, Nagelkerke's R^2=0.195; **table 3** and **figure 3a–d**). This indicates that the odds were on average five-fold lower for developing pancreatic insufficiency and CF-related liver disease and three-fold lower for developing CF-related diabetes per 1000-point increase in FIS level. As illustrated in **table 3** and **figure 3d**, age was also significantly associated with the odds of developing CF-related diabetes (adjusted OR 1.05, 95% CI 1.02–1.08; p=0.004).

Figure 3. Association of forskolin-induced swelling (FIS) with cystic fibrosis (CF)-related comorbidities



Association between residual cystic fibrosis transmembrane conductance regulator function (illustrated by steps of 1000-point change in area under the curve (AUC)) and odds of developing a) pancreatic insufficiency, b) CF-related liver disease and c) CF-related diabetes. d) In addition to FIS, age is associated with the odds of developing CF-related diabetes. Nagelkerke's R^2: pancreatic insufficiency=0.496, CF-related liver disease=0.223, CF-related diabetes=0.195.

In combination with FIS, SCC was not associated with any of the CF-related comorbidities, given the nonsignificant OR of 1 (**table 3**). Even though multiple imputation of SCC may have influenced the strength of the associations, these results suggest that FIS is more strongly associated with CF-related comorbidities than SCC when comparing both biomarkers within the same model.

Table 3. Association of forskolin-induced swelling (FIS)[#] with cystic fibrosis (CF)-related comorbidities

	Pancreatic insufficiency		CF-related liver disease		CF-related diabetes	
	Odds ratio (95% CI)	P-value	Odds ratio (95% CI)	P-value	Odds ratio (95% CI)	P-value
FIS[#]	0.18 (0.07 – 0.46)	<0.001*	0.18 (0.06 – 0.54)	0.002*	0.34 (0.12 – 0.97)	0.044*
Age	0.98 (0.93 – 1.02)	0.300	1.02 (0.99 – 1.05)	0.229	1.05 (1.02 – 1.08)	0.004*
Sex						
male	Reference category		Reference category		Reference category	
female	0.46 (0.14 – 1.46)	0.181	0.68 (0.32 – 1.44)	0.313	2.08 (0.81 – 5.37)	0.127
SCC	1.00 (0.97 – 1.04)	0.944	1.00 (0.98 – 1.02)	0.913	1.00 (0.97 – 1.04)	0.838

Adjusted odds ratios of multivariable logistic regression for pancreatic insufficiency, CF-related diabetes and CF-related liver disease. n=170. SCC: sweat chloride concentration. [#]: defined as the relative size increase of intestinal organoids (area under the curve (AUC)) after 1 h stimulation with 0.8 μM·L^{-1} forskolin, coefficient scaled 1:1000 AUC; Nagelkerke's R^2 pancreatic insufficiency=0.496, CF-related liver disease=0.223, CF-related diabetes=0.195. *: p<0.05.

DISCUSSION

This study shows that residual CFTR function quantified by FIS of patient-derived CF organoids is associated with long-term annual FEV_1pp decline and odds of developing the CF-related comorbidities pancreatic insufficiency, CF-related liver disease and CF-related diabetes, using 9-year longitudinal data of Dutch people with many distinct *CFTR* mutations and ages ranging from 0 to 61 years.

Despite the influence of genetic modifiers and other non-CFTR-dependent environmental factors on CF disease severity [1,18–20], it was remarkable to observe that *in vitro* FIS of intestinal cells has such a broad association with many nonintestinal organ systems. It illustrates that fluid secretion properties of CFTR in intestinal organoids are reflective of or related to CFTR function across many tissues.

As this study aimed to characterise *in vitro* CFTR function of many different common and rare *CFTR* mutations with FIS, the distribution of genotypes in our dataset does not correspond to the distribution of genotypes typical for the Dutch population, in which the F508del/F508del is the most common genotype. Yet it improves the generalisability of our results to the population with rare *CFTR* mutations, for which this study is especially relevant. In addition, rectal biopsies of the participants that have received modulator therapy were collected prior to the start of modulator therapy, so intestinal organoid measurements were not influenced by treatment.
Direct comparison of FIS with SCC revealed that FIS was more strongly associated with long-term multiorgan disease expression compared to SCC, which has been the most important and well-validated biomarker of CF disease until now and is a commonly used end-point to measure efficacy of CFTR-modulating drugs

[5,6]. Although the association with SCC could have been influenced by missing values and type of imputation method, the difference between FIS and SCC might also be explained by a more precise and accurate estimation of CFTR function by FIS. FIS facilitates repeated measures and is completely CFTR dependent, which reduces the impact of technological and non-CFTR biological variability in the *in vitro* assay [10,11], whereas a substantial part of variability in SCC is caused by technical and other non-CFTR-dependent biological factors [5]. Additional studies with complete datasets including repeated measurements for more precise typing of SCC are required to confirm these findings. Alternatively, it would be interesting to explore if novel sweat-based readouts that may show a higher dependency on CFTR function might also lead to better correlations with clinical observations.

In addition, FIS could be compared with other biomarkers that are being used for CF diagnosis, such as NPD and ICM. Although NPD has been used to discriminate between non-CF and CF [3,4,6–9], its ability to discriminate accurately between people with CF with differential disease progression is limited. While ICM measurements are more sensitive and have a larger dynamic range than NPD, the generation of a large dataset with repeated measures is hampered by the need for fresh rectal biopsies.

Furthermore, the data suggested that FIS has additional value in the context of disease severity association beyond the current CFTR mutation classification system. For our statistical models, we needed to prioritise one particular mutational subclass for each CFTR mutation, which is difficult due to lack of detailed experimental data for many rare (missense) mutations and the impact of potential multiple mechanistic defects for single mutations [21]. This complicates studies of mutation classification and relationship with disease severity. CFTR function by FIS demonstrated a large variability in CFTR function between participants with different genotypes, but also within genotype classes. Thus, FIS may have the potential to help to further refine patient-based classification systems beyond current genotype classification models. This might lead to more precise individual typing and prediction of disease, compared to the current classification of "mild" and "severe" CF phenotypes [22,23] or the CFTR2-based classification of mutations (CF-causing, varying clinical consequences, non-CF causing; https://cftr2.org).

Rates of annual FEV_1pp decline in this study were within the same range as reported by other recent European studies, which also showed that annual FEV_1pp decline was lower for people with CF with a "milder" disease severity as classified by genotype [24] or pancreatic status [25] and was highest in the age group between 18 and 28 years [25]. Moreover, our results are consistent with a previous

study showing a more severe CF disease phenotype in terms of pulmonary and gastrointestinal outcome parameters in infants with low FIS compared to infants with high FIS [12]. In line with our observations, *Davis et al*. [26] demonstrated that SCC by itself does not predict lung disease in people with CF.

In addition to the relationship of FIS with disease severity, several studies have shown that average FIS response to CFTR modulators correlates with short-term clinical drug response across groups with different genotypes [11,17] and in individuals with a variety of CFTR mutations [27]. Different exploratory studies did not detect an association of FIS with short-term clinical response to lumacaftor/ivacaftor in people with CF homozygous for F508del [28] or heterozygous for the A455E mutation [29] or to ivacaftor in people with residual CFTR-function mutations [30]. These studies did not demonstrate associations between FIS and biomarkers of CFTR function (NPD, SCC and ICM) [28] or FIS and SCC [29,30], nor relationships between any biomarker of CFTR function and clinical response. Additionally, treatment magnitude at group level was absent [28,29] or limited [30], suggesting that the relative impact of CFTR-dependent factors over non-CFTR-dependent factors to between-patient variations was lower as compared to the study of *Berkers et al.* [27]. This generally lowers the ability of FIS or any individual outcome to correlate after a CFTR modulator treatment. Further research in larger study populations is therefore warranted to study the association of changes in FIS or other biomarkers of CFTR function with long-term clinical effects upon CFTR modulator therapy in homogeneous and heterogeneous populations with CF.

An important limitation of this research is the retrospective observational study design. We adjusted for several confounders, but were unable to account for other prognostic factors such as pulmonary exacerbations and sputum cultures. As 34% of SCC values was missing, we used multiple imputation methods to prevent bias due to selective missing data, but this may still have influenced the associations with SCC and its comparison with FIS. Potential impact of survival bias was minimised by our subgroup and sensitivity analyses, but could not be excluded completely. Additional prospective studies should be performed to confirm the predictive value of FIS in comparison with other biomarkers such as SCC, NPD and ICM, yet our findings are in line with previous work that already demonstrated the potential of FIS as biomarker of CF disease.

In summary, this study showed that FIS of cystic fibrosis intestinal organoids is strongly associated with long-term FEV_1pp decline and odds of developing different CF-related comorbidities, suggesting that estimation of CFTR function by FIS could have important prognostic value for individual disease expression of multiple,

critical organs that are affected by CF.

Acknowledgements
We would like to thank all participants who gave informed consent for generating and testing their individual organoids and the use of their data; all members of the research teams of the Dutch CF clinics that contributed to this work; and all colleagues of the HUB Organoid Technology (Utrecht, the Netherlands) for their help with generating intestinal organoids and performing FIS experiments.

Funding
This work was funded by grants of the Dutch Cystic Fibrosis Foundation (NCFS) as part of the HIT-CF Program and by ZonMW. Funding information for this article has been deposited with the Crossref Funder Registry.

Author contributions
D. Muilwijk and E. de Poel contributed substantially to the design of the study, the acquisition, verification, analysis and interpretation of the data, and drafted the manuscript. S.W.F. Suen, A.M. Vonk, J.E. Brunsveld, E. Kruisselbrink, H. Oppelaar, M.C. Hagemeijer, P. van Mourik, G. Berkers, K.M. de Winter-de Groot, S. Heida-Michel, S.R. Jans, H. van Panhuis, M.M. van der Eerden, R. van der Meer, J. Roukema, E. Dompeling, E.**J**.M. Weersink, G.H. Koppelman, R. Vries and D.D. Zomer-van Ommen contributed to the acquisition of study data and revised the manuscript. M.J.C. Eijkemans contributed to the design of the study, analysis and interpretation of data and revised the manuscript. C.K. van der Ent and J.M. Beekman made substantial contributions to the conception and design of the study, interpretation of data and revised the manuscript.

Conflicts of interest
J.M. Beekman reports personal fees from Vertex Pharmaceuticals, Proteostasis Therapeutics, Eloxx Pharmaceuticals, Teva Pharmaceutical Industries and Galapagos, outside the submitted work; in addition, J.M. Beekman has a patent related to the FIS-assay with royalties paid. C.K. van der Ent reports grants from GSK, Nutricia, TEVA, Gilead, Vertex, ProQR, Proteostasis, Galapagos NV and Eloxx, outside the submitted work; in addition, C.K. van der Ent has a patent 10006904 with royalties paid. G.H. Koppelman reports grants from Lung Foundation of the Netherlands, Vertex Pharmaceuticals, UBBO EMMIUS foundation, GSK, TEVA the Netherlands, TETRI Foundation and European Union (H2020), outside the submitted work; and has participated in advisory board meetings for GSK and PURE-IMS outside the submitted work (money paid to institution). P. van Mourik reports financial compensation (money to institution) from Vertex for

participation in a webinar, outside the submitted work. All other authors have nothing to disclose.

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Supplementary table S1. Classification of mutations

Variant cDNA name	Variant protein name	Variant legacy name	Classification	Mutation type	CFTR2	CFTR1 reference to original report	Rationale for classification
c.948delT	p.Phe316LeufsX12	1078delT	I	Ins/del	This variant causes CF when combined with another CF-causing variant. This variant causes pancreatic insufficiency when combined with another variant that causes pancreatic insufficiency.	[1]	Class I CFTR protein synthesis defect based on sequence analysis (frameshift leading to downstream PTC) that affects a critical part of the protein and is associated with PI-CF [2].
c.1006_1007insG	p.Ile336SerfsX28	1138insG	I	Ins/del	This variant causes CF when combined with another CF-causing variant. This variant causes pancreatic insufficiency when combined with another variant that causes pancreatic insufficiency.	CFTR1 reference [3]: Schwarz M, Malone G, Super M 1992-03-16 (reference not found on Pubmed).	Class I CFTR protein synthesis defect based on sequence analysis (frameshift leading to downstream PTC) that affects a critical part of the protein and is associated with PI-CF in CFTR2.

Supplementary table S1. Continued.

Variant cDNA name	Variant protein name	Variant legacy name	Classification	Mutation type	CFTR2	CFTR1 reference to original report	Rationale for classification
c.1210-1delG	No protein name	1342-1delG	I	Splice	Not described in CFTR2.	CFTR 1 reference [3]: Huang Q, Yuan XW, Zielenski J 2008-07-11 (reference not found on Pubmed).	Class I CFTR protein synthesis defect based on sequence analysis (a splice site mutation that affects an invariant splice site sequence (+1,+2, -1,-2), leading to inproper splicing of the intron-exon boundary). Although no disease classification is present in CFTR2, we classify invariant splice site sequence variations as class I defects due to the critical impact of invariant splice mutations on splicing. This variant is described in CFTR1, but variant cDNA name and legacy name show no hits in Pubmed in the context of CF.
c.1211delG	p.Gly404AspfsX38	1343delG	I	Ins/del	This variant causes CF when combined with another CF-causing variant. This variant causes pancreatic insufficiency when combined with another variant that causes pancreatic insufficiency.	Not described in CFTR1 [3].	Class I CFTR protein synthesis defect based on sequence analysis (frameshift leading to downstream PTC) that affects a critical part of the protein and is associated with PI-CF [4].

Supplementary table S1. Continued.

Variant cDNA name	Variant protein name	Variant legacy name	Classification	Mutation type	CFTR2	CFTR1 reference to original report	Rationale for classification
c.1545_1546delTA	p.Tyr515X	1677delTA	I	Ins/del	This variant causes CF when combined with another CF-causing variant. This variant causes pancreatic insufficiency when combined with another variant that causes pancreatic insufficiency.	[5]	Class I CFTR protein synthesis defect based on sequence analysis (frameshift leading to downstream PTC) that affects a critical part of the protein and is associated with PI-CF [2].
c.1585-1G>A	No protein name	1717-1G->A	I	Splice	This variant causes CF when combined with another CF-causing variant. This variant causes pancreatic insufficiency when combined with another variant that causes pancreatic insufficiency.	[6]	Class I CFTR protein synthesis defect based on sequence analysis (frameshift leading to downstream PTC) that affects a critical part of the protein and is associated with PI-CF [2].
c.1679+1G>C	No protein name	1811+1G->C	I	Splice	This variant causes CF when combined with another CF-causing variant. This variant causes pancreatic insufficiency when combined with another variant that causes pancreatic insufficiency.	[7]	Class I CFTR protein synthesis defect based on sequence analysis (a splice site mutation that affects an invariant splice site sequence, leading to inproper splicing of the intron-exon boundary) associated with PI-CF [6].

Supplementary table S1. Continued.

Variant cDNA name	Variant protein name	Variant legacy name	Classification	Mutation type	CFTR2	CFTR1 reference to original report	Rationale for classification
c.1680-1G>A	No protein name	1812-1G->A	I	Splice	This variant causes CF when combined with another CF-causing variant. This variant causes pancreatic insufficiency when combined with another variant that causes pancreatic insufficiency.	[8]	Class I CFTR protein synthesis defect based on sequence analysis (a splice site mutation that affects an invariant splice site sequence, leading to inproper splicing of the intron-exon boundary) associated with PI-CF [2].
c.1681_1682insC	p.Val562SerfsX6	1813insC	I	Ins/del	Not described in CFTR2.	CFTR1 reference [3]: Scheffer H, Wu Y, Hofstra R, Looman M, Buys C 1996-10-23 (reference not found on Pubmed).	Class I CFTR protein synthesis defect based on sequence analysis (frameshift leading to downstream PTC) that affects a critical part of the protein.
c.1766+5G>T	No protein name	1898+5G->T	V	Splice	This variant causes CF when combined with another CF-causing variant. 67% (N=4) of patients in CFTR2 who have this variant are pancreatic insufficient.	[9]	Class V splice mutation associated with reduced wild type CFTR function based on sequence analysis (intronic variant outside invariant splice site) and associated with some PS-CF [9].

Supplementary table S1. Continued.

Variant cDNA name	Variant protein name	Variant legacy name	Classification	Mutation type	CFTR2	CFTR1 reference to original report	Rationale for classification
c.1911delG	p.Gln637HisfsX26	2043delG	I	Ins/del	This variant causes CF when combined with another CF-causing variant. This variant causes pancreatic insufficiency when combined with another variant that causes pancreatic insufficiency.	[10]	Class I CFTR protein synthesis defect based on sequence analysis (frameshift leading to downstream PTC) that affects a critical part of the protein and is associated with PI-CF in CFTR2.
c.2051_2052delAAinsG	p.Lys684SerfsX38	2183AA->G	I	ins/del	This variant causes CF when combined with another CF-causing variant. This variant causes pancreatic insufficiency when combined with another variant that causes pancreatic insufficiency.	CFTR1 reference [3]: Leoni GB, Rosatelli MC, Cao A 1994-01-13 (reference not found on pubmed).	Class I CFTR protein synthesis defect based on sequence analysis (frameshift leading to downstream PTC) that affects a critical part of the protein and is associated with PI-CF [2, 11].
c.2052delA	p.Lys684AsnfsX38	2184delA	I	Ins/del	This variant causes CF when combined with another CF-causing variant. This variant causes pancreatic insufficiency when combined with another variant that causes pancreatic insufficiency.	[12]	Class I CFTR protein synthesis defect based on sequence analysis (frameshift leading to downstream PTC) that affects a critical part of the protein and is associated with PI-CF [2].

Supplementary table S1. Continued.

Variant cDNA name	Variant protein name	Variant legacy name	Classification	Mutation type	CFTR2	CFTR1 reference to original report	Rationale for classification
c.2052_2053insA	p.Gln685ThrfsX4	2184insA	I	Ins/del	This variant causes CF when combined with another CF-causing variant. This variant causes pancreatic insufficiency when combined with another variant that causes pancreatic insufficiency.	CFTR1 reference [3]: Kalin N, Dork T, Tummler B 1992-01-02 (reference not found on Pubmed).	Class I CFTR protein synthesis defect based on sequence analysis (frameshift leading to downstream PTC) that affects a critical part of the protein and is associated with PI-CF [2, 13].
c.2657+5G>A	No protein name	2789+5G->A	V	Splice	This variant causes CF when combined with another CF-causing variant. Patients with CF who have this variant are likely to be pancreatic sufficient.	[14]	Class V splice mutation associated with reduced wild type CFTR function based on sequence analysis (intronic variant outside invariant splice site) and associated with PS-CF [2, 14].
c.2988+1G>A	No protein name	3120+1G->A	I	Splice	This variant causes CF when combined with another CF-causing variant. This variant causes pancreatic insufficiency when combined with another variant that causes pancreatic insufficiency.	[15]	Class I CFTR protein synthesis defect based on sequence analysis (a splice site mutation that affects an invariant splice site sequence, leading to inproper splicing of the intron-exon boundary) associated with PI-CF [2].

Supplementary table S1. Continued.

Variant cDNA name	Variant protein name	Variant legacy name	Classification	Mutation type	CFTR2	CFTR1 reference to original report	Rationale for classification
c.3140-26A>G	No protein name	3272-26A->G	V	Splice	This variant causes CF when combined with another CF-causing variant. Patients with CF who have this variant are likely to be pancreatic sufficient.	[10]	Class V splice mutation associated with reduced wild type CFTR function based on sequence analysis (intronic variant outside invariant splice site) and associated with PS-CF [2].
c.233_234insT	p.Trp79LeufsX32	365-366insT	I	Ins/del	This variant causes CF when combined with another CF-causing variant. This variant causes pancreatic insufficiency when combined with another variant that causes pancreatic insufficiency.	CFTR1 reference [3]: Claustres M, Altieri JP, Guittard C 2004-09-23 (reference not found on Pubmed).	Class I CFTR protein synthesis defect based on sequence analysis (frameshift leading to downstream PTC) that affects a critical part of the protein and is associated with PI-CF in CFTR2.
c.3528delC	p.Lys1177SerfsX15	3659delC	I	Ins/del	This variant causes CF when combined with another CF-causing variant. This variant causes pancreatic insufficiency when combined with another variant that causes pancreatic insufficiency.	[6]	Class I CFTR protein synthesis defect based on sequence analysis (frameshift leading to downstream PTC) that affects a critical part of the protein and is associated with PI-CF [2].

Supplementary table S1. Continued.

Variant cDNA name	Variant protein name	Variant legacy name	Classification	Mutation type	CFTR2	CFTR1 reference to original report	Rationale for classification
c.3717+12191C>T	No protein name	3849+10kbC->T	V	Splice	This variant causes CF when combined with another CF-causing variant. Patients with CF who have this variant are likely to be pancreatic sufficient.	[16]	Class V splice mutation associated with reduced wild type CFTR function based on sequence analysis (intronic variant outside invariant splice site) and associated with PS-CF [2].
c.3717+5G>T	No protein name	3849+5G->T	Unclassified	Splice	Not described in CFTR2.	Not described in CFTR1 [3].	Splice mutation that is difficult to classify due to lack of data on residual CFTR function. This variant is outside the invariant splice site and likely associated with limited wild type CFTR function (class V), but this cannot be verified as the variant cDNA name, protein name and legacy name show no hits in Pubmed in the context of CF. The mutation is also not described in CFTR1 or CFTR2.

Supplementary table S1. Continued.

Variant cDNA name	Variant protein name	Variant legacy name	Classification	Mutation type	CFTR2	CFTR1 reference to original report	Rationale for classification
c.3773_3774insT	p.Leu1258PhefsX7	3905insT	I	Ins/del	This variant causes CF when combined with another CF-causing variant. This variant causes pancreatic insufficiency when combined with another variant that causes pancreatic insufficiency.	CFTR1 reference [3]: Malik N, Hofmann S, Bosch-Al Jadooa N, Rutishauser M, Buhler E 1991-07-30 (reference not found on pubmed).	Class I CFTR protein synthesis defect based on sequence analysis (frameshift leading to downstream PTC) that affects a critical part of the protein and is associated with PI-CF [2].
c.262_263delTT	p.Leu88IlefsX22	394delTT	I	Ins/del	This variant causes CF when combined with another CF-causing variant. This variant causes pancreatic insufficiency when combined with another variant that causes pancreatic insufficiency.	[17]	Class I CFTR protein synthesis defect based on sequence analysis (frameshift leading to downstream PTC) that affects a critical part of the protein and is associated with PI-CF [2].
c.3884_3885insT	p.Ser1297PhefsX5	4016insT	I	Ins/del	This variant causes CF when combined with another CF-causing variant. This variant causes pancreatic insufficiency when combined with another variant that causes pancreatic insufficiency.	[18]	Class I CFTR protein synthesis defect based on sequence analysis (frameshift leading to downstream PTC) that affect a critical part of the protein and is associated with PI-CF [2].

Supplementary table S1. Continued.

Variant cDNA name	Variant protein name	Variant legacy name	Classification	Mutation type	CFTR2	CFTR1 reference to original report	Rationale for classification
c.4242+2T>C	No protein name	4374+2T->C	I	Splice	Not described in CFTR2.	CFTR1 reports 2 patients with suspected CF [3], unpublished.	Class I CFTR protein synthesis defect based on sequence analysis (a splice site mutation that affects an invariant splice site sequence (+1,+2, -1,-2), leading to inproper splicing of the intron-exon boundary). Although no disease classification is present in CFTR2, we classify invariant splice site sequence variations as class I defects due to the critical impact of invariant splice mutations on splicing. The variant cDNA name, protein name and legacy name show no hits in Pubmed in the context of CF.
c.4251delA	p.Glu1418ArgfsX14	4382delA	V	Ins/del	This variant causes CF when combined with another CF-causing variant. Patients with CF who have this variant are likely to be pancreatic sufficient.	[19]	Class V mutation caused by a premature stop in the late C-terminus of the CFTR protein that is associated with residual CFTR function as evident by PS-CF status [2].

Supplementary table S1. Continued.

Variant cDNA name	Variant protein name	Variant legacy name	Classification	Mutation type	CFTR2	CFTR1 reference to original report	Rationale for classification
c.1210-33_1210-6GT[13]T[4]	No protein name	5T;TG13	V	Splice	This variant has varying consequences. Some patients with this variant has CF, when combined with another CF-causing variant. Other patients with this variant do not have CF when combined with another CF-causing variant. Patients with CF who have this variant are likely to be pancreatic sufficient.	Not described in CFTR1 [3].	class V splice mutation based on sequence analysis (outside of the invariant splice sequence domain (+1,+2, -1,-2)) that affects expression level of wild type CFTR [20]. Associated with high residual CFTR function in intestinal organoids [21]. Varying clinical consequences are described in CFTR2 and following studies [22–24].
c.579+1G>T	No protein name	711+1G->T	I	Splice	This variant causes CF when combined with another CF-causing variant. This variant causes pancreatic insufficiency when combined with another variant that causes pancreatic insufficiency.	[25]	Class I CFTR protein synthesis defect based on sequence analysis (a splice site mutation that affects an invariant splice site sequence, leading to inproper splicing of the intron-exon boundary) and associated with PI-CF [2].

Supplementary table S1. Continued.

Variant cDNA name	Variant protein name	Variant legacy name	Classification	Mutation type	CFTR2	CFTR1 reference to original report	Rationale for classification
c.1364C>A	p.Ala455Glu	A455E	II	Missense	This variant causes CF when combined with another CF-causing variant. Patients with CF who have this variant are likely to be pancreatic sufficient.	[6]	Class II trafficking defect associated with normal B band and low C band, but classification is complex based on multiple observations. A445E shows comparable single channel characteristics as wild type CFTR [26] but strongly reduced C-band upon expression in heterologous expression systems and in primary epithelial CF cells, yet more C band when compared to F508del [2, 27, 28]. A455E is also responsive to VX809 or other correctors, and to potentiators [28, 29]. Based on these data, we classified the primary defect as a class II processing defect.

Supplementary table S1. Continued.

Variant cDNA name	Variant protein name	Variant legacy name	Classification	Mutation type	CFTR2	CFTR1 reference to original report	Rationale for classification
c.137C>A	p.Ala46Asp	A46D	II	Missense	This variant causes CF when combined with another CF-causing variant. Insufficient data on pancreatic status.	[30]	Class II trafficking defect based on low C band in FRT cells and no response to ivacaftor [28].
c.(1584+1_1585-1)_(1679+1_1680-1)del	No protein name	CFTRdele11	I	Ins/del	This variant causes CF when combined with another CF-causing variant. This variant causes pancreatic insufficiency when combined with another variant that causes pancreatic insufficiency.	[31]	Class I CFTR protein synthesis defect based on DNA sequence analysis (large genetic deletion) associated with PI-CF in CFTR2.
c.(2988+1_2989-1)_(3367+1_3368-1)del	No protein name	CFTRdele17a,17b	I	Ins/del	This variant causes CF when combined with another CF-causing variant. This variant causes pancreatic insufficiency when combined with another variant that causes pancreatic insufficiency.	Not described in CFTR1 [3].	Class I CFTR protein synthesis defect based on DNA sequence analysis (large genetic deletion) associated with PI-CF [2, 32].
No cDNA name	No protein name	CFTRdele19,20	I	Ins/del	Not described in CFTR2.	Not described in CFTR1 [3].	Class I CFTR protein synthesis defect based on DNA sequence analysis (large genetic deletion) associated with PI-CF [33].

Supplementary table S1. Continued.

Variant cDNA name	Variant protein name	Variant legacy name	Classification	Mutation type	CFTR2	CFTR1 reference to original report	Rationale for classification
c.54-5940_273+10250del21kb	p.Ser18ArgfsX16	CFTRdele2,3	I	Ins/del	This variant causes CF when combined with another CF-causing variant. This variant causes pancreatic insufficiency when combined with another variant that causes pancreatic insufficiency.	[34]	Class I CFTR protein synthesis defect based on DNA sequence analysis (large genetic deletion and downstream in frame PTC) associated with PI-CF [2].
c.3454G>C	p.Asp1152His	D1152H	IV	Missense	This variant has varying consequences. Some patients with this variant has CF, when combined with another CF-causing variant. Other patients with this variant do not have CF when combined with another CF-causing variant. Patients with CF who have this variant are likely to be pancreatic sufficient.	[35]	Class IV mutation associated with altered pore function. D1152H is associated with a selective bicarbonate defective (CFTRBD) conductance [2, 36].
c.178G>T	p.Glu60X	E60X	I	Nonsense	This variant causes CF when combined with another CF-causing variant. This variant causes pancreatic insufficiency when combined with another variant that causes pancreatic insufficiency.	CFTR1 reference [3]: Malone G, Schwarz M, Super M 1991-11-22 (reference not available for full access).	Class I CFTR protein synthesis defect based on sequence analysis (introduced PTC) that affects a critical part of the protein and is associated with PI-CF [2].

Supplementary table S1. Continued.

Variant cDNA name	Variant protein name	Variant legacy name	Classification	Mutation type	CFTR2	CFTR1 reference to original report	Rationale for classification
c.2188G>T	p.Glu730X	E730X	I	Nonsense	Not described in CFTR2.	[37]	Class I CFTR protein synthesis defect based on sequence analysis (introduced PTC) that affects a critical part of the protein.
c.274G>A	p.Glu92Lys	E92K	II	Missense	This variant causes CF when combined with another CF-causing variant. 44% (N=17) of patients in CFTR2 who have this variant are pancreatic insufficient.	[38]	Class II trafficking defect associated with strongly reduced CFTR maturation (C band) in heterologous cells systems [39, 40], and no response to ivacaftor [28]. Strong rescue by VX809 [2, 41].
c.1521_1523delCTT	p.Phe508del	F508del	II	Ins/del	This variant causes CF when combined with another CF-causing variant. This variant causes pancreatic insufficiency when combined with another variant that causes pancreatic insufficiency.	[42–44]	A class II trafficking defect associated with strongly reduced CFTR maturation (c band) in heterologous and primary cells systems which is considered dominant over additional defects that lower gating and surface retention[29, 39, 40, 45], associated with PI-CF [2].

Supplementary table S1. Continued.

Variant cDNA name	Variant protein name	Variant legacy name	Classification	Mutation type	CFTR2	CFTR1 reference to original report	Rationale for classification
c.3745G>A	p.Gly1249Arg	G1249R	Unclassified	Missense	This variant causes CF when combined with another CF-causing variant. 57% (N=4) of patients in CFTR2 who have this variant are pancreatic insufficient.	[46]	Organoids from G1249R/F508del showed prominent in vitro response to VX770 and not VX809, and clinical response to treatment with ivacaftor was observed [21]. It is likely a gating mutation based on ivacaftor sensitivity, but it might also have class IV defects. No papers could be found which experimentally characterize protein function. We therefore categorized this mutation as unclassified.
c.532G>A	p.Gly178Arg	G178R	III	Missense	This variant causes CF when combined with another CF-causing variant. 75% (N=57) of patients in CFTR2 who have this variant are pancreatic insufficient.	[25]	Ivacaftor responsive gating mutation associated with normal C band expression in heterologous expression models [47] and associated with PI-CF [2].

Supplementary table S1. Continued.

Variant cDNA name	Variant protein name	Variant legacy name	Classification	Mutation type	CFTR2	CFTR1 reference to original report	Rationale for classification
c.1381G>A	p.Gly461Arg	G461R	Unclassified	Missense	Not described in CFTR2.	Not described in CFTR1 [3].	F508del/G461R shows response to ivacaftor therapy both in vitro (organoids) and in vivo [48], however no papers have been published that experimentally characterize CFTR-protein. We therefore categorized this mutation as unclassified.
c.1624G>T	p.Gly542X	G542X	I	Nonsense	This variant causes CF when combined with another CF-causing variant. This variant causes pancreatic insufficiency when combined with another variant that causes pancreatic insufficiency.	[6]	Class I CFTR protein synthesis defect based on sequence analysis (introduced PTC) that affects a critical part of the protein and is associated with PI-CF [2].
c.1648G>T	p.Gly550X	G550X	I	Nonsense	This variant causes CF when combined with another CF-causing variant. This variant causes pancreatic insufficiency when combined with another variant that causes pancreatic insufficiency.	CFTR1 reference [3]: Deiman C, Deelan W, Halley D 1992-02-25 (reference not found on Pubmed)	Class I CFTR protein synthesis defect based on sequence analysis (introduced PTC) that affects a critical part of the protein and is associated with PI-CF.

Supplementary table S1. Continued.

Variant cDNA name	Variant protein name	Variant legacy name	Classification	Mutation type	CFTR2	CFTR1 reference to original report	Rationale for classification
c.1882G>A	p.Gly628Arg	G628R	II	Missense	This variant causes CF when combined with another CF-causing variant. Patients with CF who have this variant are likely to be pancreatic sufficient.	[10]	Class II trafficking defect associated with strongly reduced C band expression in heterologous expression model and rescue by the corrector miglustat [49]. Based on these results we have classified this mutation as class II.
c.254G>A	p.Gly85Glu	G85E	II	Missense	This variant causes CF when combined with another CF-causing variant. This variant causes pancreatic insufficiency when combined with another variant that causes pancreatic insufficiency.	[25]	Class II trafficking defect associated with strongly reduced C band expression in heterologous expression model and no response to ivacaftor [2, 28].
c.2908G>C	p.Gly970Arg	G970R	I	Splice	This variant causes CF when combined with another CF-causing variant. This variant causes pancreatic insufficiency when combined with another variant that causes pancreatic insufficiency.	[37]	Fidler et al. showed that the G>C change in the last position of the canonical 5′ splice donor site of exon 17 weakens the likelihood that this position will be recognized as a splice donor site and showed evidence that the G970R mutation must be reclassified primarily as a splice mutation [50], in contrast with previous work suggesting a gating defect [51].

Supplementary table S1. Continued.

Variant cDNA name	Variant protein name	Variant legacy name	Classification	Mutation type	CFTR2	CFTR1 reference to original report	Rationale for classification
c.3080T>C	p.Ile1027Thr	I1027T	Unclassified	Missense	This variant does not cause CF when combined with another CF-causing variant. There may patients in the CFTR2 database with this variant who have CF, but this variant is not the cause of their CF.	[10]	Non-CF causing polymorphism that was present here in cis with F508del which designated the complex allele as class II [2].
c.1007T>A	p.Ile336Lys	I336K	II	Missense	This variant causes CF when combined with another CF-causing variant. Patients with CF who have this variant are likely to be pancreatic sufficient.	[37]	Class II trafficking defect associated with strongly reduced C band expression in heterologous expression model and no response to ivacaftor [2, 28].
c.1519_1521delATC	p.Ile507del	I507del	II	Ins/del	This variant causes CF when combined with another CF-causing variant. This variant causes pancreatic insufficiency when combined with another variant that causes pancreatic insufficiency.	[52]	Class II trafficking defect associated with strongly reduced C band expression in heterologous expression model [2, 53].

Supplementary table S1. Continued.

Variant cDNA name	Variant protein name	Variant legacy name	Classification	Mutation type	CFTR2	CFTR1 reference to original report	Rationale for classification
No cDNA name	No protein name	IVS11-1G->C	I	Splice	Not described in CFTR2.	Not described in CFTR1 [3].	Class I CFTR protein synthesis defect based on sequence analysis (a splice site mutation that affects an invariant splice site sequence, leading to inproper splicing of the intron-exon boundary).
No cDNA name	p.Leu1034Pro	L1034P	Unclassified	Missense	Not described in CFTR2.	Not described in CFTR1 [3].	Unclassified. Variant cDNA name, protein name and legacy name show no hits in Pubmed in the context of CF. The mutation is also not described in CFTR1 or CFTR2.
c.4004T>C	p.Leu1335Pro	L1335P	Unclassified	Missense	This variant causes CF when combined with another CF-causing variant. 61% (N=11) of patients in CFTR2 who have this variant are pancreatic insufficient.	CFTR1 reference [3]: Zielenski J, Tzountzouris J, Tsui L-C 1997-08-12 (reference not found on pubmed)	Unclassified. Mutation is listed as responsive to symdeko or trikafta (www.symdeko.com or www.trikafta.com).
c.617T>G	p.Leu206Trp	L206W	II	Missense	This variant causes CF when combined with another CF-causing variant. Patients with CF who have this variant are likely to be pancreatic sufficient.	[19]	Class II trafficking defect associated with strongly reduced C band expression in heterologous expression model and no response to ivacaftor [2, 28].

Supplementary table S1. Continued.

Variant cDNA name	Variant protein name	Variant legacy name	Classification	Mutation type	CFTR2	CFTR1 reference to original report	Rationale for classification
c.2195T>G	p.Leu732X	L732X	I	Nonsense	This variant causes CF when combined with another CF-causing variant. This variant causes pancreatic insufficiency when combined with another variant that causes pancreatic insufficiency.	CFTR1 reference [3]: Malone G, Haworth A, Schwartz M 1994-10-05 (reference not available for full access)	Class I CFTR protein synthesis defect based on sequence analysis (introduced PTC) that affects a critical part of the protein and is associated with PI-CF [2].
c.2780T>C	p.Leu927Pro	L927P	Unclassified	Missense	This variant causes CF when combined with another CF-causing variant. This variant causes pancreatic insufficiency when combined with another variant that causes pancreatic insufficiency.	[54]	Unclassified. L927P may cause cystic fibrosis by interfering with conformational changes necessary for channel opening [55]. The surface expression level of the L927P mutant is 43% that of the wild-type protein, but its channel activity is only 0.1% [28]. Others reported a normal protein expression [2].

Supplementary table S1. Continued.

Variant cDNA name	Variant protein name	Variant legacy name	Classification	Mutation type	CFTR2	CFTR1 reference to original report	Rationale for classification
c.3909C>G	p.Asn1303Lys	N1303K	II	Missense	This variant causes CF when combined with another CF-causing variant. This variant causes pancreatic insufficiency when combined with another variant that causes pancreatic insufficiency.	[56]	Class II trafficking defect associated with strongly reduced C band expression in both heterologous and primary CF cells [29, 57]. It does not or hardly responds to ivacaftor or lumacaftor alone or in combination, but responds to VX-445 [2, 58].
c.4046delG	p.Gly1349AlafsX5	No legacy name	Unclassified	Ins/del	Not described in CFTR2.	Not described in CFTR1 [3].	CFTR protein synthesis defect, but due to the late position of the stopcodon it is unclear whether the resulting protein has some associated function.
c.4243-3T>A	No protein name	No legacy name	Unclassified	Splice	Not described in CFTR2.	CFTR1 reports obstructive lung function, bronciectasis and infections. Pancreatic sufficiency, no liver abnormalities, elevated sweat chloride level [3], but unpublished.	Variant cDNA name, protein name and legacy name show no hits in Pubmed in the context of CF. The mutation is also not described in CFTR2. It is unclear whether the splice defect can be classified as class I or V.

Supplementary table S1. Continued.

Variant cDNA name	Variant protein name	Variant legacy name	Classification	Mutation type	CFTR2	CFTR1 reference to original report	Rationale for classification
No cDNA name	p.Gln1012Pro	Q1012P	Unclassified	Missense	Not described in CFTR2.	Not described in CFTR1 [3].	Unclassified. Variant cDNA name, protein name and legacy name show no hits in Pubmed in the context of CF. The mutation is also not described in CFTR1 or CFTR2.
c.1477C>T	p.Gln493X	Q493X	I	Nonsense	This variant causes CF when combined with another CF-causing variant. This variant causes pancreatic insufficiency when combined with another variant that causes pancreatic insufficiency.	[6]	Class I CFTR protein synthesis defect based on sequence analysis (introduced PTC) that affects a critical part of the protein and is associated with PI-CF [2].
c.3196C>T	p.Arg1066Cys	R1066C	II	Missense	This variant causes CF when combined with another CF-causing variant. This variant causes pancreatic insufficiency when combined with another variant that causes pancreatic insufficiency.	[10]	Class II trafficking defect associated with strongly reduced C band expression in heterologous cell expression systems and no response to ivacaftor [2, 28, 59].

Supplementary table S1. Continued.

Variant cDNA name	Variant protein name	Variant legacy name	Classification	Mutation type	CFTR2	CFTR1 reference to original report	Rationale for classification
c.3197G>A	p.Arg1066His	R1066H	II	Missense	This variant causes CF when combined with another CF-causing variant. This variant causes pancreatic insufficiency when combined with another variant that causes pancreatic insufficiency.	[60]	Class II trafficking defect associated with strongly reduced C band expression in heterologous cell expression systems and no response to ivacaftor [2, 28].
c.3484C>T	p.Arg1162X	R1162X	I	Nonsense	This variant causes CF when combined with another CF-causing variant. This variant causes pancreatic insufficiency when combined with another variant that causes pancreatic insufficiency.	[61]	Class I CFTR protein synthesis defect based on sequence analysis (introduced PTC) that affects a critical part of the protein and is associated with PI-CF [2].

Supplementary table S1. Continued.

Variant cDNA name	Variant protein name	Variant legacy name	Classification	Mutation type	CFTR2	CFTR1 reference to original report	Rationale for classification
c.350G>A	p.Arg117His	R117H	IV	Missense	This variant has varying consequences. Some patients with this variant have CF, when combined with another CF-causing variant. Other patients with this variant do not have CF when combined with another CF-causing variant. Patients with CF who have this variant are likely to be pancreatic sufficient.	[62]	R117H is associated with altered conductance properties and was originally classified as class IV [63]. It is a complex allele associated with a intronic polyT tract (5T, 7T or 9T) that affects splicing efficiency which associates with disease severity (class V). Others have also reported trafficking defects [64] or gating defects [65]. C-band expression in heterologous systems is mostly reflective of wt CFTR. R117H shows acute responsiveness to ivacaftor in heterologous systems and primary cells but only limited response to correctors [21]. For this study, we retain the original classification of R117H as class IV mutation [2].

Supplementary table S1. Continued.

Variant cDNA name	Variant protein name	Variant legacy name	Classification	Mutation type	CFTR2	CFTR1 reference to original report	Rationale for classification
c.4074A>T	p.Arg1358Ser	R1358S	Unclassified	Missense	Not described in CFTR2.	CFTR1 reference [3]: Férec C 1999-01-01 (reference not found on Pubmed).	Unclassified. Variant cDNA name, protein name and legacy name show no hits in Pubmed in the context of CF. The mutation is also not described in CFTR2.
c.1000C>T	p.Arg334Trp	R334W	IV	Missense	This variant causes CF when combined with another CF-causing variant. Patients with CF who have this variant are likely to be pancreatic sufficient.	[66]	Normal CFTR protein expression in heterologous system but altered single channel conductance characteristic of class IV [63]. Some response to ivacaftor but not lumacaftor in primary CF cells [2, 21].

Supplementary table S1. Continued.

Variant cDNA name	Variant protein name	Variant legacy name	Classification	Mutation type	CFTR2	CFTR1 reference to original report	Rationale for classification
c.1040G>C	p.Arg347Pro	R347P	II	Missense	This variant causes CF when combined with another CF-causing variant. 64% (N=302) of patients in CFTR2 who have this variant are pancreatic insufficient.	[62]	Class II trafficking defect associated with reduced C band expression in heterologous cell expression systems. R347P was originally found to have altered channel conductance properties, but also matures very inefficiently (c-band ~15% of wild-type and is not associated with function despite some expression [28]. Moreover, (F508del/R347P) shows no clear detectable response to ivacaftor in primary CF cells [21]. Others also found processing defects [39]. This supports a primary defect in processing (class II). The limited product that reaches the surface has likely altered channel properties [2].

Supplementary table S1. Continued.

Variant cDNA name	Variant protein name	Variant legacy name	Classification	Mutation type	CFTR2	CFTR1 reference to original report	Rationale for classification
c.1657C>T	p.Arg553X	R553X	I	Nonsense	This variant causes CF when combined with another CF-causing variant. This variant causes pancreatic insufficiency when combined with another variant that causes pancreatic insufficiency.	[67]	Class I CFTR protein synthesis defect based on sequence analysis (introduced PTC) that affects a critical part of the protein and is associated with PI-CF [2].
c.221G>C	p.Arg74Pro	R74P	Unclassified	Missense	Not described in CFTR2.	Not described in CFTR1 [3].	Unclassified. Variant cDNA name, protein name and legacy name show no hits in Pubmed in the context of CF. The mutation is also not described in CFTR1 or CFTR2.
c.2290C>T	p.Arg764X	R764X	I	Nonsense	This variant causes CF when combined with another CF-causing variant. This variant causes pancreatic insufficiency when combined with another variant that causes pancreatic insufficiency.	[15]	Class I CFTR protein synthesis defect based on sequence analysis (introduced PTC) that affects a critical part of the protein and is associated with PI-CF [2].

Supplementary table S1. Continued.

Variant cDNA name	Variant protein name	Variant legacy name	Classification	Mutation type	CFTR2	CFTR1 reference to original report	Rationale for classification
c.2353C>T	p.Arg785X	R785X	I	Nonsense	This variant causes CF when combined with another CF-causing variant. This variant causes pancreatic insufficiency when combined with another variant that causes pancreatic insufficiency.	[68]	Class I CFTR protein synthesis defect based on sequence analysis (introduced PTC) that affects a critical part of the protein and is associated with PI-CF in CFTR2.
c.3752G>A	p.Ser1251Asn	S1251N	III	Missense	This variant causes CF when combined with another CF-causing variant. 72% (N=84) of patients in CFTR2 who have this variant are pancreatic insufficient.	[69]	Classified as ivacaftor responsive class III gating mutation with normal C-band expression [2, 47].
c.53G>T	p.Ser18Ile	S18I	Unclassified	Missense	Not described in CFTR2.	Not described in CFTR1 [3].	Unclassified. Variant cDNA name, protein name and legacy name show no hits in Pubmed in the context of CF. The mutation is also not described in CFTR1 or CFTR2.

Supplementary table S1. Continued.

Variant cDNA name	Variant protein name	Variant legacy name	Classification	Mutation type	CFTR2	CFTR1 reference to original report	Rationale for classification
c.1466C>A	p.Ser489X	S489X	I	Nonsense	This variant causes CF when combined with another CF-causing variant. This variant causes pancreatic insufficiency when combined with another variant that causes pancreatic insufficiency.	CFTR1 reference [3]: Macdonald K, Haworth, A Malone G, Schwarz M 1994-08-15 (reference not found on Pubmed)	Class I CFTR protein synthesis defect based on sequence analysis (introduced PTC) that affects a critical part of the protein and is associated with PI-CF [2, 70].
c.4186A>C	p.Thr1396Pro	T1396P	Unclassified	Missense	Not described in CFTR2.	Not described in CFTR1 [3].	Unclassified. One study reported no consistent manifestations of CF over time [71].
c.3477delT	p.Val1160X	V1160X	I	Nonsense	Not described in CFTR2.	[72]	Class I CFTR protein synthesis defect based on sequence analysis (introduced PTC) that affects a critical part of the protein.
c.3846G>A	p.Trp1282X	W1282X	I	Nonsense	This variant causes CF when combined with another CF-causing variant. This variant causes pancreatic insufficiency when combined with another variant that causes pancreatic insufficiency.	[73]	Class I CFTR protein synthesis defect based on sequence analysis (introduced PTC) that affects a critical part of the protein and is associated with PI-CF [2].

Supplementary table S1. Continued.

Variant cDNA name	Variant protein name	Variant legacy name	Classification	Mutation type	CFTR2	CFTR1 reference to original report	Rationale for classification
c.2036G>A	p.Trp679X	W679X	I	Nonsense	Not described in CFTR2.	CFTR1 reference [3]: Walker C, Tsui L-C, Zielenski J 1999-09-27 (reference not found on Pubmed).	Class I CFTR protein synthesis defect based on sequence analysis (introduced PTC) that affects a critical part of the protein.
c.2537G>A	p.Trp846X	W846X	I	Nonsense	This variant causes CF when combined with another CF-causing variant. This variant causes pancreatic insufficiency when combined with another variant that causes pancreatic insufficiency.	[74]	Class I CFTR protein synthesis defect based on sequence analysis (introduced PTC) that affects a critical part of the protein and is associated with PI-CF [2].
c.3276C>A	p.Tyr1092X	Y1092X	I	Nonsense	This variant causes CF when combined with another CF-causing variant. This variant causes pancreatic insufficiency when combined with another variant that causes pancreatic insufficiency.	[75]	Class I CFTR protein synthesis defect based on sequence analysis (introduced PTC) that affects a critical part of the protein and is associated with PI-CF [2].

Supplementary table S1. Continued.

Variant cDNA name	Variant protein name	Variant legacy name	Classification	Mutation type	CFTR2	CFTR1 reference to original report	Rationale for classification
c.325T>G	p.Tyr109Asp	Y109D	Unclassified	Missense	Not described in CFTR2.	Not described in CFTR1 [3].	Unclassified. One report [76] described mutation as CF-causing, elevated sweat chloride levels and pancreatic insufficiency. No characterization of protein function has been published.
c.2547C>A	p.Tyr849X	Y849X	I	Nonsense	This variant causes CF when combined with another CF-causing variant. This variant causes pancreatic insufficiency when combined with another variant that causes pancreatic insufficiency.	[77]	Class I CFTR protein synthesis defect based on sequence analysis (introduced PTC) that affects a critical part of the protein and is associated with PI-CF in CFTR2.

Overview of the individual mutations in cDNA, protein and legacy name (according to the human genome variation society (HGVS) nomenclature) and corresponding CFTR classification including the rationale behind classification. The rationale is based on available literature and the clinical consequence of the mutation found in the CFTR2 database. In addition to the rationale, the original report describing the mutation for the first time was added to the table, derived from the CFTR1 database.

Supplementary table S2. Individual genotypes of study participants

ID	Genotype (legacy name)	Genotype classification	Mutation group
1	G542X/CFTRdele2.3	Class I	ins/del-nonsense
2	1811+1G>C/1811+1G>C	Class I	splice-splice
3	1717-1G>A/2183AA>G	Class I	ins/del-splice
4	W1282X/W1282X	Class I	nonsense-nonsense
5	G542X/W679X	Class I	nonsense-nonsense
6	1811+1G>C/1811+1G>C	Class I	splice-splice
7	R785X/R785X	Class I	nonsense-nonsense
8	1717-1G>A/3905insT	Class I	ins/del-splice
9	R1162X/3659delC	Class I	ins/del-nonsense
10	1811+1G>C/1811+1G>C	Class I	splice-splice
11	711+1G>T/CFTRdele11	Class I	ins/del-splice
12	1677delTA/3120+1G>A	Class I	ins/del-splice
13	L732X/L732X	Class I	nonsense-nonsense
14	1811+1G>C/1811+1G>C	Class I	splice-splice
15	711+1G>T/711+1G>T	Class I	splice-splice
16	F508del/L206W	Class II	ins/del-missense
17	F508del/G628R	Class II	ins/del-missense
18	F508del/I336K	Class II	ins/del-missense
19	A455E/3659delC	Class II	ins/del-missense
20	A455E/1343delG	Class II	ins/del-missense
21	R1066H/CFTRdele2.3	Class II	ins/del-missense
22	F508del/G628R	Class II	ins/del-missense
23	A455E/E60X	Class II	missense-nonsense
24	F508del/G628R	Class II	ins/del-missense
25	F508del/F508del	Class II	ins/del-ins/del
26	G542X/R1066C	Class II	missense-nonsense
27	F508del/2184delA	Class II	ins/del-ins/del
28	F508del/R347P	Class II	ins/del-missense
29	F508del/Y1092X	Class II	ins/del-nonsense
30	F508del/365-366insT(W79fs)	Class II	ins/del-ins/del
31	F508del/R1066C	Class II	ins/del-missense
32	F508del/1342-1delG	Class II	ins/del-splice
33	F508del/W846X	Class II	ins/del-nonsense
34	F508del/1717-1G>A	Class II	ins/del-splice
35	F508del/CFTRdele17a.17b	Class II	ins/del-ins/del
36	F508del/F508del	Class II	ins/del-ins/del
37	R1066C/R1066H	Class II	missense-missense
38	V1160X/E92K	Class II	missense-nonsense
39	F508del/1078delT	Class II	ins/del-ins/del
40	F508del/W1282X	Class II	ins/del-nonsense
41	F508del/R347P	Class II	ins/del-missense
42	F508del/1078delT	Class II	ins/del-ins/del
43	F508del/F508del	Class II	ins/del-ins/del
44	F508del/F508del	Class II	ins/del-ins/del
45	N1303K/G550X	Class II	missense-nonsense
46	F508del/CFTRdele19.20	Class II	ins/del-ins/del
47	F508del/F508del	Class II	ins/del-ins/del

Supplementary table S2. Continued.

ID	Genotype (legacy name)	Genotype classification	Mutation group
48	F508del/R347P	Class II	ins/del-missense
49	F508del/I336K	Class II	ins/del-ins/del
50	A46D/A46D	Class II	missense-missense
51	F508del/3659delC	Class II	ins/del-ins/del
52	F508del/CFTRdele17a.17b	Class II	ins/del-ins/del
53	F508del/E730X	Class II	ins/del-nonsense
54	F508del/2183AA>G	Class II	ins/del-ins/del
55	F508del/Y1092X	Class II	ins/del-nonsense
56	F508del/1813insC	Class II	ins/del-ins/del
57	F508del/E60X	Class II	ins/del-nonsense
58	F508del/711+1G>T	Class II	ins/del-splice
59	F508del/Y1092X	Class II	ins/del-nonsense
60	F508del/G85E	Class II	ins/del-missense
61	F508del/I507del	Class II	ins/del-ins/del
62	F508del/1717-1G>A	Class II	ins/del-splice
63	F508del/W1282X	Class II	ins/del-nonsense
64	F508del/3659delC	Class II	ins/del-ins/del
65	F508del/711+1G>T	Class II	ins/del-splice
66	F508del/1717-1G>A	Class II	ins/del-splice
67	F508del/CFTRdele17a.17b	Class II	ins/del-ins/del
68	F508del/Y849X	Class II	ins/del-nonsense
69	F508del/1717-1G>A	Class II	ins/del-splice
70	N1303K/G85E	Class II	missense-missense
71	F508del/CFTRdele2.3	Class II	ins/del-ins/del
72	F508del/CFTRdele17a.17b	Class II	ins/del-ins/del
73	A46D/A46D	Class II	missense-missense
74	F508del/Y849X	Class II	ins/del-nonsense
75	F508del/G85E	Class II	ins/del-missense
76	F508del/S489X	Class II	ins/del-nonsense
77	F508del/2184delA	Class II	ins/del-ins/del
78	G542X/R1066C	Class II	missense-nonsense
79	F508del/711+1G>T	Class II	ins/del-splice
80	F508del/F508del	Class II	ins/del-ins/del
81	F508del/W1282X	Class II	ins/del-nonsense
82	F508del/Y1092X	Class II	ins/del-nonsense
83	F508del/N1303K	Class II	ins/del-missense
84	F508del/711+1G>T	Class II	ins/del-splice
85	I507del/4374+2T->C	Class II	ins/del-splice
86	F508del/711+1G>T	Class II	ins/del-splice
87	F508del/R1162X	Class II	ins/del-nonsense
88	F508del/G550X	Class II	ins/del-nonsense
89	F508del/G550X	Class II	ins/del-nonsense
90	F508del/F508del	Class II	ins/del-ins/del
91	F508del/F508del	Class II	ins/del-ins/del
92	F508del/Q493X	Class II	ins/del-nonsense
93	F508del/4016insT	Class II	ins/del-ins/del
94	F508del/394delTT	Class II	ins/del-ins/del

Supplementary table S2. Continued.

ID	Genotype (legacy name)	Genotype classification	Mutation group
95	F508del/IVS11-1G>C	Class II	ins/del-splice
96	F508del/G550X	Class II	ins/del-nonsense
97	F508del/R1162X	Class II	ins/del-nonsense
98	F508del/R1162X	Class II	ins/del-nonsense
99	F508del/Y1092X	Class II	ins/del-nonsense
100	F508del/N1303K	Class II	ins/del-missense
101	F508del/W1282X	Class II	ins/del-nonsense
102	F508del/2184insA	Class II	ins/del-ins/del
103	F508del/F508del	Class II	ins/del-ins/del
104	F508del/R1162X	Class II	ins/del-nonsense
105	F508del/E60X	Class II	ins/del-nonsense
106	F508del/R1162X	Class II	ins/del-nonsense
107	F508del/S1251N	Class III	ins/del-missense
108	F508del/S1251N	Class III	ins/del-missense
109	F508del/S1251N	Class III	ins/del-missense
110	F508del/S1251N	Class III	ins/del-missense
111	F508del/S1251N	Class III	ins/del-missense
112	F508del/S1251N	Class III	ins/del-missense
113	F508del/S1251N	Class III	ins/del-missense
114	F508del/S1251N	Class III	ins/del-missense
115	S1251N/1717-1G>A	Class III	missense-splice
116	F508del/S1251N	Class III	ins/del-missense
117	F508del/G178R	Class III	ins/del-missense
118	3905insT/D1152H	Class IV	ins/del-missense
119	F508del/D1152H	Class IV	ins/del-missense
120	W1282X/R117H;7T	Class IV	missense-nonsense
121	F508del/R117H;7T/9T	Class IV	ins/del-missense
122	R1162X/D1152H	Class IV	missense-nonsense
123	R117H;7T/R553X	Class IV	missense-nonsense
124	R334W/N1303K	Class IV	missense-missense
125	R334W/R334W	Class IV	missense-missense
126	D1152H/R1162X	Class IV	missense-nonsense
127	R334W/R764X	Class IV	missense-nonsense
128	F508del/5T;TG13	Class V	ins/del-splice
129	F508del/5T;TG13	Class V	ins/del-splice
130	G542X/3849+10kbC>T	Class V	nonsense-splice
131	F508del/5T;TG13	Class V	ins/del-splice
132	A455E/5T;TG13	Class V	missense-splice
133	F508del/3849+10kbC>T	Class V	ins/del-splice
134	F508del/3849+10kbC>T	Class V	ins/del-splice
135	F508del/3849+10kbC>T	Class V	ins/del-splice
136	3272-26A>G/3272-26A>G	Class V	splice-splice
137	F508del/3849+10kbC>T	Class V	ins/del-splice
138	3272-26A>G/G970R	Class V	splice-splice
139	F508del/3272-26A>G	Class V	ins/del-splice
140	F508del/3272-26A>G	Class V	ins/del-splice
141	F508del/3272-26A>G	Class V	ins/del-splice

Supplementary table S2. Continued.

ID	Genotype (legacy name)	Genotype classification	Mutation group
142	3272-26A>G/1898+5G>T	Class V	splice-splice
143	F508del/3272-26A>G	Class V	ins/del-splice
144	4382delA/2043delG	Class V	ins/del-ins/del
145	F508del/4382delA	Class V	ins/del-ins/del
146	F508del/2789+5G>A	Class V	ins/del-splice
147	F508del/4382delA	Class V	ins/del-ins/del
148	Y849X/2789+5G>A	Class V	nonsense-splice
149	1078delT/3272-26A>G	Class V	ins/del-splice
150	3849+10kbC>T/1717-1G>A	Class V	splice-splice
151	F508del/c.4243-3T>A	Unclassified	ins/del-splice
152	F508del/R1358S	Unclassified	ins/del-missense
153	F508del;I1027T/UNK	Unclassified	ins/del-unknown
154	UNK/UNK	Unclassified	unknown-unknown
155	R553X/c.4243-3T>A	Unclassified	nonsense-splice
156	F508del/T1396P	Unclassified	ins/del-missense
157	F508del/G461R	Unclassified	ins/del-missense
158	N1303K/Q1012P	Unclassified	missense-missense
159	F508del/UNK	Unclassified	ins/del-unknown
160	R117H;7T/UNK	Unclassified	missense-unknown
161	F508del/G1249R	Unclassified	ins/del-missense
162	UNK/UNK	Unclassified	unknown-unknown
163	F508del/G1249R	Unclassified	ins/del-missense
164	F508del/UNK	Unclassified	ins/del- unknown
165	F508del/3849+5G>T	Unclassified	ins/del-splice
166	L1335P/L1335P	Unclassified	missense-missense
167	F508del/R74P	Unclassified	ins/del-missense
168	F508del/L1034P	Unclassified	ins/del-missense
169	F508del/S18I	Unclassified	ins/del-missense
170	F508del/Y109D	Unclassified	ins/del-missense
171	W1282X/L927P	Unclassified	missense-nonsense
172	F508del/UNK	Unclassified	ins/del-unknown
173	F508del/c.4046delG	Unclassified	ins/del-ins/del

Overview of individual genotypes with corresponding CFTR mutation classification according to the rationale described in supplementary table 1. Genotypes are provided in legacy name, unless stated otherwise (c. = cDNA code). Study participants were categorized into one mutation class based on the mildest of both mutation classes, or to unclassified when one of the mutation classes was unknown or uncertain. Mutation group was defined by the combination of mutation types of both alleles.

Supplementary table S3. Association of FIS with FEV_1pp decline in subgroup analysis 4-25 years

N=107, obs=644	Coefficient (95% CI)	P-value
Age	-1.57 (-2.03 – -1.10)	<0.001*
FIS	-3.01 (-11.07 – 5.04)	0.462
FIS*age	0.49 (0.03 – 0.96)	0.039*
Treatment		
none	Reference category	
ivacaftor	9.63 (4.93 – 14.33)	<0.001*
lumacaftor/ivacaftor	-4.32 (-10.70 – 2.06)	0.184
Sex		
male	Reference category	
female	0.16 (-6.38 – 6.71)	0.961
Genotype class		
unclassified	Reference category	
class I	0.93 (-14.29 – 16.16)	0.904
class II	6.21 (-6.30 – 18.72)	0.330
class III	7.86 (-6.99 – 22.71)	0.299
class IV	21.37 (1.52 – 41.22)	0.349
class V	-1.58 (-20.64 – 17.47)	0.870
SCC	-0.09 (-0.25 – 0.07)	0.264

Regression coefficients of linear mixed effects model for FEV_1pp within a subgroup including participants between 4-25 years of age.

Abbreviations and definitions: FEV_1pp: forced expiratory volume in 1 s % predicted. Age in years. FIS: Forskolin-induced swelling, defined as the relative size increase of intestinal organoids (AUC) after 1h stimulation with 0.8 μM/L forskolin, coefficient scaled 1:1000 AUC. SCC: Sweat chloride concentration in mmol/L. FIS*age indicates the difference in annual FEV_1pp decline per 1000 AUC change in FIS level. Genotype class: CFTR protein function class of the mildest of both CFTR mutations. * Significance level $P < 0.05$.

Supplementary table S4. Association of FIS with FEV_1pp decline in sensitivity analysis including genotype group

	N=138, obs=970		Subgroup: N=100, obs=601	
	Coefficient (95% CI)	**P-value**	**Coefficient (95% CI)**	**P-value**
Age	-1.25 (-1.54 – -0.96)	<0.001*	-1.68 (-2.15 – -1.21)	<0.001*
FIS	-2.93 (-8.42 – 2.56)	0.295	-5.22 (-12.15 – 1.71)	0.140
FIS*age	0.36 (0.12 – 0.61)	0.004*	0.69 (0.17 – 1.20)	0.009*
Treatment				
none	Reference category		Reference category	
ivacaftor	8.43 (4.79 – 12.06)	<0.001*	10.03 (4.88 – 15.17)	<0.001*
lumacaftor/ivacaftor	-3.44 (-7.89 – 1.00)	0.129	-4.30 (-10.66 – 2.06)	0.185
Sex				
male	Reference category		Reference category	
female	-0.07 (-6.21 – 6.07)	0.982	0.82 (-5.72 – 7.36)	0.805
Genotype group				
ins/del – missense	Reference category		Reference category	
ins/del – nonsense	-0.24 (-9.98 – 9.51)	0.962	2.05 (-7.94 – 12.05)	0.687
ins/del – splice	-2.91 (-12.08 – 6.25)	0.533	-2.29 (-11.89 – 7.31)	0.640
ins/del – ins/del	-0.45 (-10.05 – 9.14)	0.927	1.56 (-8.72 – 11.84)	0.766
missense – nonsense	7.85 (-6.51 – 22.21)	0.284	8.40 (-8.39 – 25.19)	0.326
missense – missense	8.77 (-5.46 – 23.00)	0.227	13.84 (-1.12 – 28.79)	0.070
splice – splice	-6.30 (-21.46 – 8.87)	0.415	1.74 (-15.01 – 18.50)	0.838
SCC	-0.09 (-0.24 – 0.07)	0.289	-0.12 (-0.29 – 0.05)	0.176

Regression coefficients of linear mixed effects model for FEV_1pp with genotype group and subgroup only including participants between 4 – 25 years of age.

Abbreviations and definitions: FEV_1pp: forced expiratory volume in 1 s % predicted. Age in years. FIS: Forskolin-induced swelling, defined as the relative size increase of intestinal organoids (AUC) after 1h stimulation with 0.8 µM/L forskolin, coefficient scaled 1:1000 AUC. FIS*age indicates the difference in annual FEV_1pp decline per 1000 AUC change in FIS level. SCC: Sweat chloride concentration in mmol/L. Genotype group: combination of CFTR mutation types on both alleles. * Significance level $P < 0.05$.

Supplementary table S5. Association of SCC with FEV_1pp decline

N=149, obs=1054	Coefficient (95% CI)	P-value
Age	--0.26 (-1.12 – 0.61)	0.563
FIS	1.78 (-4.05 – 7.62)	0.549
SCC	0.004 (-0.19 – 0.20)	0.971
SCC*age	-0.01 (-0.02 – 0.002)	0.121
Treatment		
none	Reference category	
ivacaftor	8.04 (4.61 – 11.47)	<0.001*
lumacaftor/ivacaftor	-3.98 (-8.44 – 0.49)	0.081
Sex		
male	Reference category	
female	-0.75 (-6.83 – 5.32)	0.807
Genotype class		
unclassified	Reference category	
class I	0.73 (-13.51 – 14.97)	0.920
class II	5.37 (-5.62 – 16.36)	0.338
class III	10.66 (-3.45 – 24.76)	0.138
class IV	12.11 (-4.46 – 28.69)	0.152
class V	0.23 (-14.60 – 15.06)	0.976

Abbreviations and definitions: FEV_1pp: forced expiratory volume in 1 s % predicted. Age in years. FIS: Forskolin-induced swelling, defined as the relative size increase of intestinal organoids (AUC) after 1h stimulation with 0.8 μM/L forskolin, coefficient scaled 1:1000 AUC. SCC: Sweat chloride concentration in mmol/L. FIS*age indicates the difference in annual FEV_1pp decline per 1000 AUC change in FIS level. Genotype class: CFTR protein function class of the mildest of both CFTR mutations. * Significance level P < 0.05.

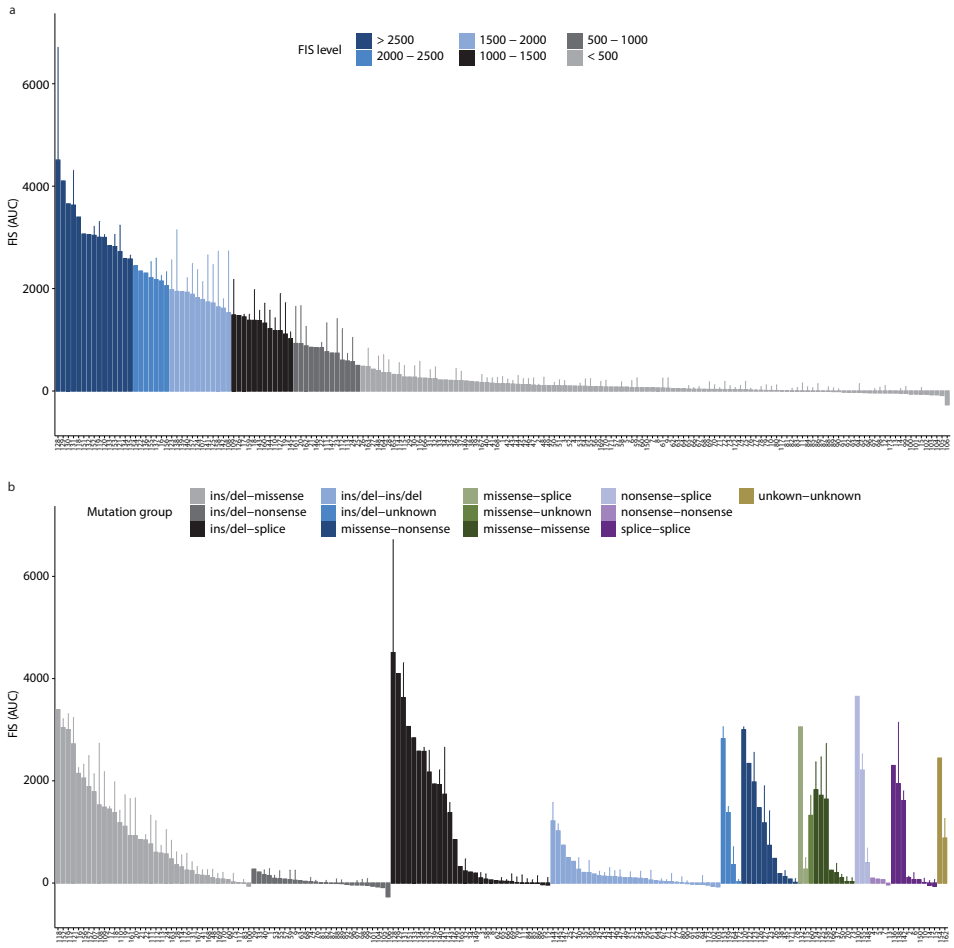
Supplementary table S6. Comparison of the association between FEV_1pp decline and FIS versus SCC

N=149, obs=1054	Coefficient (95% CI)	P-value
Age	-1.16 (-2.36 – 0.03)	0.056
FIS	-2.42 (-9.15 – 4.30)	0.480
FIS*age	0.33 (0.05 – 0.60)	0.020*
SCC	-0.09 (-0.31 – 0.12)	0.392
SCC*age	0.00 (-0.01 – 0.01)	0.995
Treatment		
none	Reference category	
ivacaftor	8.02 (4.61 – 11.43)	<0.001*
lumacaftor/ivacaftor	-3.75 (-8.20 – 0.70)	0.098
Sex		
male	Reference category	
female	-0.99 (-7.06 – 5.07)	0.748
Genotype class		
unclassified	Reference category	
class I	0.51 (-13.65 – 14.67)	0.944
class II	5.34 (-5.60 – 16.27)	0.339
class III	10.25 (-3.82 – 24.32)	0.153
class IV	11.09 (-5.44 – 27.63)	0.188
class V	-2.45 (-17.33 – 12.43)	0.747

Abbreviations and definitions: FEV_1pp: forced expiratory volume in 1 s % predicted. Age in years. FIS: Forskolin-induced swelling, defined as the relative size increase of intestinal organoids (AUC) after 1h stimulation with 0.8 μM/L forskolin, coefficient scaled 1:1000 AUC. SCC: Sweat chloride concentration in mmol/L. FIS*age indicates the difference in annual FEV_1pp decline per 1000 AUC change in FIS level. SCC*age indicates the difference in annual FEV_1pp decline per 1-unit change in SCC level. Genotype class: CFTR protein function class of the mildest of both CFTR mutations. * Significance level P < 0.05.

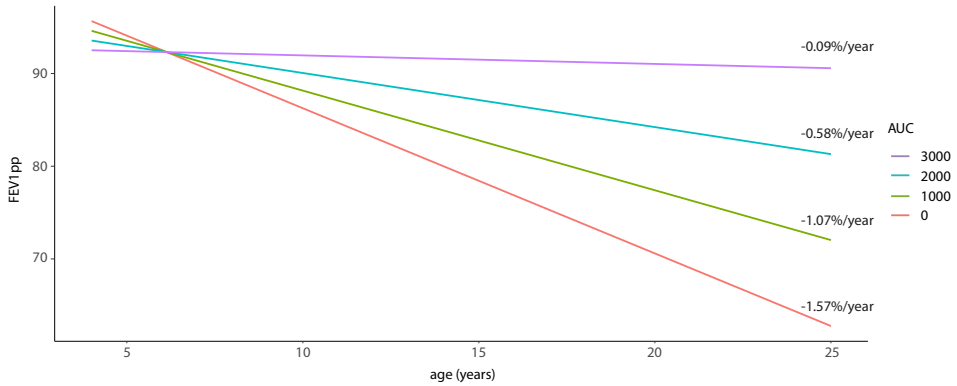
SUPPLEMENTARY **Figures.**

Supplementary figure S1. Individual FIS responses



a) waterfall plot of FIS responses stimulated with 0.8 μM forskolin for 1 hour of all study participants. b) waterfall plot of FIS responses at 0.8 μM forskolin per mutation group. Groups were defined by the combination of the mutation type of both mutations. Bars represent mean+SD of replicates, ranging from n=2 to n=7. The numbers on the x-axes represent the participant number and correspond to the numbers in figure 1b-c. Genotypes are specified in **supplementary table S2**.

Supplementary figure S2. Association of FIS with long-term FEV_1pp decline in subgroup 4-25 years



Predicted FEV_1pp decline based on model coefficients in **supplementary table S3**, illustrating the association between different levels of residual CFTR function and long-term FEV_1pp decline in the subgroup analysis. The analysis was performed with FIS as continuous variable, yet for illustrative purposes predicted FEV_1pp decline is plotted by steps of 1000 AUC from 4 to 25 years, reflecting the age range of the subgroup. Average predicted annual FEV_1pp decline per AUC level is specified on the right.

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CHAPTER 4

Long-term effectiveness of dual CFTR modulator treatment of Cystic Fibrosis

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ERJ Open Research 2022, Nov 14;8(4):00204-2022

ABSTRACT

Background

Although short-term efficacy of lumacaftor/ivacaftor and tezacaftor/ivacaftor is clearly established in clinical trials, data on long-term effectiveness is limited. This registry-based cohort study assessed real-world longitudinal outcomes of F508del-homozygous people with cystic fibrosis (pwCF) ⩾12 years, up to 3 years after the introduction of dual cystic fibrosis transmembrane conductance regulator (CFTR) modulators.

Methods

Annual data (2010–2019) were retrieved from the Dutch Cystic Fibrosis Registry. Longitudinal trends of per cent predicted forced expiratory volume in 1 s (FEV_1 % pred) decline, body mass index (BMI), BMI Z-score and intravenous antibiotic treatment duration before and after CFTR modulator initiation were assessed with linear and negative binomial mixed models.

Results

We included 401 participants (41.9% female, baseline age 24.5 years (IQR: 18.0–31.5 years), mean±SD baseline FEV_1 70.5±23.4% pred). FEV_1 decline improved from −1.36% pred per year to −0.48% pred per year after modulator initiation (change: 0.88% pred, 95% CI: 0.35–1.39%, p=0.001). This change was even 1.40% pred per year (95% CI: −0.0001–2.82%, p=0.050) higher in participants with baseline FEV_1 <40% pred. In adults, annual BMI trend was not altered (change: 0.10 $kg \cdot m^{-2} \cdot year^{-1}$, 95% CI:−0.01–0.21, p=0.079). Annual BMI Z-score in children reversed from −0.08 per year before modulator treatment to 0.06 per year afterwards (change: 0.14 per year, 95% CI: 0.06–0.22, p<0.001). Intravenous antibiotic treatment duration showed a three-fold reduction in the first year after modulator initiation (incidence rate ratios (IRR): 0.28, 95% CI: 0.19–0.40, p<0.001), but the annual trend did not change in the subsequent years (IRR: 1.19, 95% CI: 0.94–1.50, p=0.153).

Conclusion

Long-term effectiveness of dual CFTR modulator therapies on FEV_1 decline, BMI and intravenous antibiotic treatment duration is less pronounced in a real-world setting than in clinical trials and varies considerably between pwCF and different baseline FEV_1 levels.

INTRODUCTION

Over the last decade, the treatment landscape of cystic fibrosis (CF) has drastically changed with the arrival of cystic fibrosis transmembrane conductance regulator (CFTR) modulators [1]. Lumacaftor/ ivacaftor (LUM/IVA) and tezacaftor/ivacaftor (TEZ/IVA) were the first two dual therapies that became available for people with CF (pwCF) who are homozygous for the F508del mutation. Lumacaftor and tezacaftor are small molecules that enhance the processing and trafficking of mature CFTR protein to the cell membrane [2], whereas ivacaftor augments the channel opening probability [3]. The first phase 3 randomised controlled trials (RCTs) that supported the licensing of LUM/IVA were conducted in pwCF homozygous for F508del older than 12 years of age with a baseline per cent predicted forced expiratory volume in 1 s (FEV_1 % pred) between 40% and 90%. These RCTs demonstrated a mean absolute improvement of 2.6–4% pred FEV_1, an increase in body mass index (BMI) and a reduction of pulmonary exacerbation rate and intravenous (*i.v.*) antibiotic use after 24 weeks of treatment [4]. A few years later, phase 3 RCTs with TEZ/ IVA showed a comparable short-term efficacy, albeit with substantially less side-effects than LUM/IVA [5].

Subsequently, the original phase 3 open-label extension trials provided the first evidence of long-term efficacy of LUM/IVA and TEZ/IVA. These trials showed a mean estimated FEV_1 decline between −1.3% pred and −0.8% pred per year after 120 weeks of CFTR modulator treatment, compared to −2.3% pred to −2.1% pred in matched historical controls. Furthermore, the absolute change from baseline BMI continued to increase whereas pulmonary exacerbation rate and *i.v.* antibiotic use remained substantially lower [6,7]

Especially in chronic diseases like CF, collection of long-term data on the effectiveness of new treatments is important, given the strictly controlled conditions and inclusion criteria as well as a relatively short follow-up in RCTs [8]. Currently, real-world evidence of the long-term benefits after the first year of treatment with LUM/IVA and TEZ/IVA is still limited. No real-world studies have been published yet that include a large group of pwCF homozygous for F508del with different ages and disease stages, covering important clinical outcomes after 1 year of CFTR modulator treatment. Patient registries such as the Dutch Cystic Fibrosis Registry (NCFR), which is part of the European Medicines Agency (EMA)-approved European Cystic Fibrosis Society Patient Registry (ECFSPR), play a key role in the acquisition of long-term real-world evidence of new treatments.
In this study, we aimed to assess real-world longitudinal changes in FEV_1 decline, BMI and annual duration of *i.v.* antibiotic treatment in people with CF homozygous

for F508del, up to 3 years after the introduction of the dual CFTR modulating therapies LUM/IVA and TEZ/IVA, using NCFR data.

MATERIALS AND METHODS

Study design and population

In this registry-based observational cohort study, we used longitudinal data from the NCFR between 2010 and 2019. The NCFR retrospectively collects annualised clinical data of pwCF who are treated in one of the seven Dutch CF centres and who provided informed consent for the collection and use of their data for research. This nationwide informed consent procedure is part of an agreement between the Dutch CF Foundation and the Dutch CF centres, which was approved by the local Institutional Review Boards (IRBs) when the NCFR was initiated. The use of clinical data for this research project was considered as exempt from the Dutch Act for Medical Research Involving Human Subjects by the IRB of the University Medical Center Utrecht, the Netherlands, and was approved by the NCFR Steering Group. The NCFR covers 95% of pwCF in The Netherlands and is part of the EMA-approved ECFSPR. All Dutch pwCF homozygous for F508del aged 12 years and older who received LUM/IVA treatment before January 2018 were eligible for this study, regardless of a transition to TEZ/IVA or treatment discontinuation, either temporary or permanent. Participant data were censored after lung transplantation, death or lost to follow-up. No exclusion criteria were specified.

Study parameters

Longitudinal changes in FEV_1 % pred, BMI, BMI Z-score and annual duration of *i.v.* antibiotic treatment after commencement with LUM/IVA were considered as clinical outcomes. The NCFR collects annual best FEV_1 % pred measurements, calculated according to the Global Lung function Initiative (GLI) guideline [9], which were used to assess the mean annual change in FEV_1 % pred before and after CFTR modulator initiation. Annual weight and height measurements were used to calculate BMI in adults of 19 years and older, whereas BMI Z-scores standardised for age and sex were calculated according to the World Health Organisation (WHO) Growth Reference for children below 19 years [10]. Duration of annual *i.v.* antibiotic treatment was calculated in total number of days per year. Baseline was defined by the first start date of LUM/IVA as registered in the NCFR. If applicable, date of transition to TEZ/IVA was collected. CFTR modulator treatment status at each measurement timepoint was dichotomised as treatment=no before baseline and treatment=yes after baseline. Data regarding sex, age and presence of *Pseudomonas aeruginosa* and *Staphylococcus aureus* in annual sputum cultures were also collected.

Statistical analysis
Descriptive statistics were used to summarise baseline characteristics of the study population.

A linear mixed effects model was used to assess longitudinal trends in FEV_1 % pred before and after CFTR modulator initiation. Following the same approach, linear mixed model analyses of BMI and BMI Z-score were performed in data subsets including measurements at an age above and below 19 years, respectively. Changes in the annual duration of *i.v.* antibiotic treatment were analysed with a negative binomial mixed effects model. Detailed model specifications are provided in the supplementary material.

To facilitate a comparison of real-world data with data from controlled registration trials, subgroup analyses in participants with a baseline FEV_1 between 40% and 90% pred were performed for each model. For FEV_1 and *i.v.* antibiotic treatment duration, we also compared longitudinal trends of participants with a baseline FEV_1 <40% and ⩾90% pred to the group with a FEV_1 between 40% and 90% pred at baseline and between adults >18 years and adolescents of 12–18 years. This was not performed for BMI and BMI Z-score because these subgroups were already divided by age category according to the WHO reference standard and were therefore too small to allow for a subgroup analysis with multiple baseline FEV_1 groups. Finally, additional subgroup analyses were conducted for each model to compare longitudinal and acute changes after CFTR modulator treatment between participants who transitioned to TEZ/IVA and participants who continued with LUM/IVA and between females and males.

To adjust for potential confounders, age and sex were included as covariates in the models, where appropriate. The proportion of missing data was highest for the annual duration of *i.v.* antibiotic treatment (32.2%), followed by 4.1% of FEV_1 % pred measurements, 0.5% of BMI Z-scores in children <19 years and 0.2% of BMI in adults ⩾19 years.

To adjust for missing data, all models with FEV_1 % pred, BMI and BMI Z-score as outcomes were assessed using Bayesian methods which allow for a joint imputation and analysis of incomplete datasets. Changes in the duration of *i.v.* antibiotic treatment were analysed using maximum likelihood estimation methods without imputation of missing data, which is a robust method for missing outcome data.

Estimations of the Bayesian models were displayed as coefficients with corresponding 95% confidence intervals and p-values. P-values <0.05 were considered statistically

significant. Statistical packages jointAI and lme4 of R for Mac version 4.1.1 were used for the analyses.

RESULTS

Study population

A total of 401 pwCF with the F508del/F508del mutation were included in this study. Baseline characteristics are summarised in **table 1**. Median follow-up time before and after CFTR modulator initiation was 7.9 years (IQR: 7.5–7.9 years) *versus* 2.1 years (IQR: 2.1–2.2 years), respectively. Censoring occurred in 13 (3.2%) participants due to lung transplantation (n=11) or death (n=2). Approximately half (51.9%) of the study population transitioned from LUM/IVA to TEZ/IVA between 2018 and 2019, after mean±SD 2.0±0.6 years of initial LUM/IVA treatment. Last measured FEV_1 before CFTR modulator initiation was between 40% and 90% pred in 257 (64.1%) of the participants.

Table 1. Baseline characteristics

CFTR modulator treatment, n (%)	
Lumacaftor/ivacaftor (LUM/IVA)	401 (100)
Transition to tezacaftor/ivacaftor (TEZ/IVA)	208 (51.9)
Time (years) to transition from LUM/IVA to TEZ/IVA, mean (SD)	2.0 (0.6)
Death, n (%)	2 (0.5)
Lung transplantation, n (%)	11 (2.7)
Sex, n (%)	
Male	233 (58.1)
Female	168 (41.9)
Age (years), median (IQR)	24.5 (18.0 – 31.5)
Age 12-18 years, n (%)	116 (28.9)
Age > 18 years, n (%)	285 (71.1)
Missing, n (%)	0
ppFEV1pp (%), mean (SD)	70.5 (23.4)
ppFEV1 <40%, n (%)	51 (12.7)
ppFEV1 40-70%, n (%)	128 (31.9)
ppFEV1 70-90%, n (%)	129 (32.2)
ppFEV1 ≥90%, n (%)	90 (22.4)
Missing, n (%)	3 (0.8)
BMI adults (kg/m^2) ≥ 19 years, mean (SD)	21.4 (2.5)
Missing, n (%)	5 (1.8)
BMI Z-score children 12-19 years, mean (SD)	-0.5 (0.8)
Missing, n (%)	0
Received intravenous antibiotic treatment, n (%)	
Yes	149 (37.3)
No	201 (50.0)
Missing	51 (12.7)
Duration of intravenous antibiotic treatments (days), median (IQR)	23 (17 – 42)

Table 1. Continued

***Pseudomonas Aeruginosa* sputum culture status, n (%)**	
Positive	179 (44.6)
Negative	209 52.2)
Missing	13 (3.2)
***Staphylococcus Aureus* sputum culture status, n (%)**	
Positive	196 (48.9)
Negative	192 (47.9)
Missing	13 (3.2)
Cystic Fibrosis-related diabetes, n (%)	
Yes	156 (38.9)
No	234 (58.4)
Missing	11 (2.7)
Cystic Fibrosis-related liver disease, n (%)	
Yes	89 (22.2)
No	255 (63.6)
Missing	57 (14.2)

Abbreviations: BMI: body mass index. CFTR: Cystic fibrosis transmembrane conductance regulator. ppFEV1: percent predicted forced expiratory volume in 1s.
Definitions: age was calculated at the date of CFTR modulator initiation (baseline). ppFEV1, BMI, BMI Z-score, number and duration of received intravenous antibiotic treatment, *Pseudomonas Aeruginosa* and *Staphylococcus Aureus* sputum culture status, CF-related diabetes and CF-related liver disease status reported at the last annual measurement preceding CFTR modulator initiation. The median duration of intravenous treatments was calculated for the 149 participants who received intravenous antibiotics in the last year prior to CFTR modulator initiation.

Lung function decline

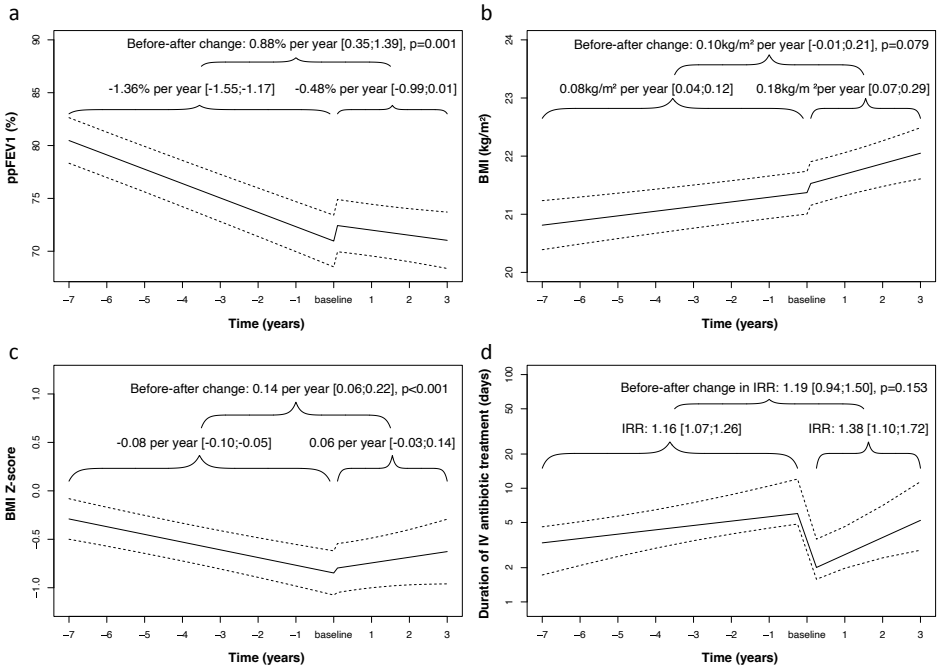
Overall, we observed a moderate acute change in the estimated FEV_1 at baseline (FEV_1 at baseline: 70.97% pred, 95% CI: 68.52–73.42%) after CFTR modulator initiation (change: 1.51% pred, 95% CI: 0.56–2.46%, p=0.002). The mean annual FEV_1 decline improved from −1.36% pred per year to −0.48% pred per year after CFTR modulator initiation (change: 0.88% pred, 95% CI: 0.35–1.39%, p=0.001; **figure 1a** and **table 2**).

Table 2. Bayesian linear mixed effects model estimates of ppFEV1 (n=401, Years of observation=3844)

	Unadjusted coefficient	95% CI	P-value	Adjusted coefficient#	95% CI	P-value
Intercept	69.09	66.78 – 71.39	<0.001	70.97	68.52 – 73.42	<0.001
Time	-1.35	-1.54 – -1.15	<0.001*	-1.36	-1.55 – -1.17	<0.001*
CFTR modulator	1.51	0.49 – 2.48	0.002*	1.51	0.56 – 2.46	0.002*
Time : CFTR Modulator	0.86	0.31 – 1.41	0.002*	0.88	0.35 – 1.39	0.001*

Interpretation: the intercept represents the average ppFEV1 of the study population at the time of CFTR modulator initiation (baseline). The coefficient of time (in years) reflects the average annual ppFEV1 decline in the years before CFTR modulator initiation. The coefficient CFTR modulator indicates the acute change in average ppFEV1 after CFTR modulator initiation, whereas time : CFTR modulator represents the change in annual ppFEV1 decline in the years after CFTR modulator initiation compared to the years before. # Coefficients were adjusted for the main effects of sex, age at baseline and the interaction effect of age at baseline with time. *Significance level p<0.05.

Figure 1. Longitudinal time trends of clinical outcomes before and after CFTR modulator initiation



Estimated longitudinal trends of percent predicted forced expiratory volume in 1s (ppFEV1), body mass index (BMI), BMI Z-score and annual intravenous (IV) antibiotic treatment duration. Time ranges from -7 years before to +3 years after CFTR modulator initiation, with time=0 (baseline) defined by the start date of CFTR modulator treatment. Dashed lines represent 95% confidence intervals, which are also shown between square brackets.
Panel 1a: average ppFEV1 decline before CFTR modulator treatment was -1.36% per year (95% CI: -1.55;-1.17%), which changed with 0.88% per year (95% CI: 0.35;1.39%, p=0.001) after CFTR modulator initiation (**table 2**). The calculated ppFEV1 decline after modulator initiation (-0.48% per year, 95% CI: -0.99;0.01%) was added to the figure to illustrate the difference in ppFEV1 decline before and after CFTR modulator initiation. Panel 1b: in adults ≥ 19 years, BMI gradually increased over time with 0.08 kg/m^2 per year (95% CI: 0.04;0.12 kg/m^2) before CFTR modulator treatment. This annual BMI trend did not significantly change (change: 0.10 kg/m^2 per year (95% CI: -0.01;0.21 kg/m^2, p=0.079) in the years after modulator initiation (**table 3**). The calculated BMI after modulator initiation (0.18 kg/m^2 per year, 95% CI: 0.07;0.29 kg/m^2) was added to the figure to illustrate the difference in BMI before and after CFTR modulator initiation. Panel 1c: In children < 19 years, BMI Z-score initially decreased over time before CFTR modulator initiation, with an average of -0.08 per year (95% CI: -0.10;-0.05). This annual trend significantly changed into an increasing trend (change: 0.14 per year (95% CI: 0.06;0.22, p<0.001) in the years after CFTR modulator initiation (**table 4**). The calculated BMI Z-score after modulator initiation (0.06 per year, 95% CI: 0.03;0.14) was added to the figure to illustrate the difference in BMI Z-score before and after CFTR modulator initiation. Panel 1d: the average annual duration of IV antibiotic treatment (in days) increased with 16% (IRR: 1.16, 95% CI: 1.07;1.26, p<0.001) in the years preceding CFTR modulator treatment. In the year of CFTR modulator initiation, a drop in the average duration of IV antibiotics was observed, leading to a three-times lower (IRR 0.28, 95% CI: 0.19 – 0.40, p<0.001) duration of IV antibiotic treatment compared to the years before CFTR modulator initiation. In the years after CFTR modulator initiation, the annual average duration of IV treatment did not significantly change (change in IRR: 1.19, 95% CI: 0.94;1.50, p=0.153; **table 5**) The calculated IRR after modulator initiation (IRR: 1.84, 95% CI: 1.10;1.72) was added to the figure to illustrate the trend after CFTR modulator initiation.

The acute impact of CFTR modulator treatment was slightly higher in the subgroup of participants with a baseline FEV_1 between 40% and 90% pred, with an acute change from baseline FEV_1 of 2.59% pred (95% CI: 1.40–3.78%, p<0.001; **supplementary**

table S1a). The magnitude of change in FEV_1 decline was comparable to the change in the entire cohort (change: 0.81% pred per year, 95% CI: 0.11–1.50%, p=0.026; **supplementary table S1a** and **supplementary figure S1a**).

In participants with a baseline FEV_1 <40% pred, the acute improvement in FEV_1 was not significantly different from those with a FEV_1 40%–90% pred before CFTR modulator initiation (difference: −1.24% pred, 95% CI: −4.25–1.78%, p=0.420; **supplementary table S1a**). As illustrated in **supplementary figure S1b**, the mean change in FEV_1 decline after CFTR modulator initiation was even 1.40% pred per year higher (95% CI: −0.0001–2.82%, p=0.050; **supplementary table S1a**) than in the participants with a baseline FEV_1 40%–90% pred.

In the group with baseline FEV_1 ⩾90% pred, a decline of FEV_1 was not observed (**supplementary table S1a**). Additional subgroup analyses did not show any differences in acute or longitudinal FEV_1 changes after CFTR modulator initiation between participants who transitioned to TEZ/IVA or continued LUM/IVA treatment, between females and males or between adults and adolescents **(supplementary tables S1b–d**).

BMI and BMI Z-scores

In adults of 19 years and older, estimated baseline BMI (21.37 $kg{\cdot}m^{-2}$, 95% CI: 21.00–21.74 $kg{\cdot}m^{-2}$) did not show an acute change after CFTR modulator initiation (change: 0.08 $kg{\cdot}m^{-2}$, 95% CI: −0.34– 0.31 $kg{\cdot}m^{-2}$, p=0.097; **table 3**). As illustrated in **figure 1b**, the increasing annual BMI trend prior to modulator initiation (0.08 $kg{\cdot}m^{-2}$ per year, 95% CI: 0.04–0.12 $kg{\cdot}m^{-2}$, p<0.001) was not significantly altered after CFTR modulator initiation (change: 0.10 $kg{\cdot}m^{-2}$ per year, 95% CI: −0.01–0.21 $kg{\cdot}m^{-2}$, p=0.079; **table 3**).

Table 3. Bayesian linear mixed effects model estimates of BMI in adults ≥ 19 years (n=312, Years of observation=2317)

	Unadjusted coefficient	95% CI	P-value	Adjusted coefficient#	95% CI	P-value
Intercept	21.40	21.12 – 21.67	<0.001	21.37	21.00 – 21.74	<0.001
Time	0.06	0.03 – 0.31	<0.001*	0.08	0.04 – 0.12	<0.001*
CFTR modulator	0.14	-0.02 – 0.31	0.086	0.14	-0.03 – 0.31	0.097
Time : CFTR Modulator	0.06	-0.03 – 0.15	0.217	0.10	-0.01 – 0.21	0.079

Interpretation: the intercept represents the average BMI at the time of CFTR modulator initiation (baseline) in adults of 19 years and older. The coefficient of time indicates the average annual change in BMI in the years before modulator initiation. The coefficient of CFTR modulator reflects the acute change in BMI after modulator initiation, whereas time : CFTR modulator represents the change in annual BMI in the years after CFTR modulator initiation compared to the years before. # Coefficients were adjusted for the main effects of sex, age at baseline, the interaction effect of age at baseline with time and the interaction effect of age at baseline with time and CFTR modulator treatment. * Significance level p<0.05.

The subgroup analysis in participants with a baseline FEV_1 between 40% and 90% pred showed similar longitudinal trends, with a change in annual BMI of 0.13 $kg \cdot m^{-2}$ (95% CI: −0.04–0.32 $kg \cdot m^{-2}$, p=0.058) after CFTR modulator initiation (**supplementary table S2a** and **supplementary figure S2a**). In addition, no significant differences were demonstrated in acute or longitudinal changes after CFTR modulator initiation in participants who transitioned to TEZ/IVA compared to participants who continued LUM/IVA treatment (**supplementary table S2b**) or between females and males (**supplementary table S2c**).

Following WHO growth reference standards [10], BMI Z-scores were calculated for children with an age at baseline of 12–18 years. Estimated BMI Z-score at baseline −0.85 (95% CI: −0.08 – −0.62) did not show an acute change after modulator initiation (change: 0.05, 95% CI: −0.10–0.19, p=0.537; **table 4**). **Figure 1c** shows that the annual trend of BMI Z-score improved with 0.14 per year (95% CI: 0.06–0.22, p<0.001) to 0.06 per year in children below 19 years of age, which was in contrast with the decreasing trend prior to CFTR modulating treatment (−0.08 per year, 95% CI: −0.10 – −0.05, p<0.001; **table 4**).

Trends of BMI Z-score in the subgroup with a baseline FEV_1 between 40% and 90% pred were similar to the overall trends, although the longitudinal change after CFTR modulator initiation was slightly smaller compared to the entire cohort (change: 0.09 per year, 95% CI: −0.02–0.20, p=0.113; **supplementary table S3a** and **supplementary figure S2b**). Again, no significant differences were observed in acute or longitudinal changes after CFTR modulator initiation between participants who transitioned to TEZ/IVA and participants who continued LUM/IVA treatment (**supplementary table S3b**). The mean acute improvement of BMI Z-score after CFTR modulator initiation was 0.33 (95% CI: 0.06–0.61, p=0.018) higher in females compared to males, whereas longitudinal trends were comparable between sexes (**supplementary table S3c**).

Intravenous antibiotic treatment duration

In the first year after CFTR modulator initiation, the mean duration of *i.v.* antibiotic treatment became approximately three times lower (incidence rate ratio (IRR): 0.28, 95% CI: 0.19–0.40, p<0.001) than the mean 4.38 days (95% CI: 2.82–6.79 days) in the last year preceding CFTR modulator initiation (**table 5**). In contrast, the mean annual duration of received *i.v.* antibiotics was not significantly altered after CFTR modulator initiation (IRR 1.19, 95% CI: 0.94–1.50, p=0.153), which increased with 16% per year (IRR 1.16, 95% CI: 1.07–1.26, p<0.001) in the years before CFTR modulator initiation (**table 5** and **figure 1d**).

Table 4. Bayesian linear mixed effects model estimates of BMI Z-score in children < 19 years (n=225, Years of observation=1552)

	Unadjusted coefficient	95% CI	P-value	Adjusted coefficient#	95% CI	P-value
Intercept	-0.60	-0.73 – -0.47	<0.001	-0.85	-1.08 – -0.62	<0.001
Time	-0.06	-0.09 – -0.05	<0.001*	-0.08	-0.11 – -0.05	<0.001*
CFTR modulator	0.003	-0.15 – 0.15	0.959	0.05	-0.10 – 0.19	0.537
Time : CFTR Modulator	0.13	0.05 – 0.21	0.002*	0.14	0.06 – 0.22	<0.001*

Interpretation: the intercept represents the average BMI Z-score at the time of CFTR modulator initiation (baseline) in children under 19 years (according to WHO growth reference standards). The coefficient of time indicates the average annual change in BMI Z-score in the years before modulator initiation. The coefficient of CFTR modulator reflects the acute change in BMI Z-score after modulator initiation, whereas time : CFTR modulator represents the change in annual BMI Z-score in the years after CFTR modulator initiation compared to the years before. # Coefficients were adjusted for the main effects of sex, age at baseline, the interaction effect of sex with time and the interaction effect of age at baseline with time. * Significance level p<0.05.

Table 5. Negative binomial mixed effects model estimates of the duration of IV antibiotic treatment (n=364, Years of observation=2805)

	Unadjusted coefficient	IRR	95% CI (IRR)	P-value	Adjusted coefficient#	IRR	95% CI (IRR)	P-value
Intercept	1.76	5.83	3.97 – 8.56	<0.001	1.48	4.38	2.82 – 6.79	<0.001
Time	0.15	1.16	1.07 – 1.26	<0.001*	0.15	1.16	1.07 – 1.26	<0.001*
CFTR modulator	-1.28	0.28	0.19 – 0.40	<0.001*	-1.28	0.28	0.19 – 0.40	<0.001*
Time : CFTR Modulator	0.16	1.18	0.93 – 1.49	0.170	0.17	1.19	0.94 – 1.50	0.153

Interpretation: coefficients are on the log-scale. Incidence rate ratios (IRR) are transformed back to the original scale. The IRR of the intercept represents the average duration of received IV antibiotics (in days) of the study population at the time of CFTR modulator initiation (baseline). The IRR of time shows the relative annual change in the duration of IV antibiotics before CFTR modulator treatment. The IRR of CFTR modulator reflects the acute change in the duration of IV antibiotics in the first year after CFTR modulator initiation, whereas time : CFTR modulator treatment indicates the relative change of IV antibiotic treatment in the years after modulator initiation compared to the annual trend before CFTR modulator use. # Coefficients and IRRs were adjusted for sex and age at baseline. * Significance level p<0.05.

In the subgroup of participants with baseline FEV_1 40%–90% pred, the mean duration of received *i.v.* antibiotics in the last year preceding CFTR modulator initiation was slightly higher (6.16 days, 95% CI: 5.32–15.38 days), whereas the longitudinal changes before and after modulator initiation were comparable to the overall results (**supplementary table S4a** and **supplementary figure S3a**). As shown in **supplementary figure S3b**, trends of participants with a baseline FEV_1 <40% pred were comparable to participants with baseline FEV_1 40%–90% pred, but the mean *i.v.* antibiotic treatment duration in participants with a FEV_1 ⩾90% pred at baseline was considerably lower and did not increase after CFTR modulator initiation (**supplementary table S4a**). Additional subgroup analyses did not show differences between participants who transitioned to TEZ/IVA or who continued LUM/IVA treatment, between females and males or between adults and adolescents (**supplementary tables S4b–d**).

DISCUSSION

This study provided real-world data of the long-term effectiveness of LUM/IVA and TEZ/IVA on important pulmonary outcomes and nutritional status, covering almost 4000 patient-years of observation in pwCF homozygous for F508del, up to 3 years after the introduction of these dual CFTR modulating therapies. Although the pivotal RCTs and open-label extension trials demonstrated a clear efficacy of LUM/IVA and TEZ/IVA on several clinical end-points in pwCF with a baseline FEV_1 between 40% and 90% pred [4–7], our results emphasised that real-world effectiveness is less pronounced, with considerable differences in long-term trends among pwCF and a FEV_1 below 40% pred or above 90% pred upon CFTR modulator initiation.

Real-world improvement of annual FEV_1 decline was slightly lower than the 1% pred change in FEV_1 decline estimated by the long-term open-label extension trial data [6,7]. This was demonstrated by a mean change of 0.81% pred and 0.88% pred per year after CFTR modulator initiation in both the subgroup with baseline FEV_1 40%–90% pred and in the entire cohort, respectively. In contrast with the short-term trials [4,5], the acute change of FEV_1 after modulator initiation was limited in the entire cohort. However, we did observe an acute improvement of 2.59% pred in the subgroup of participants with a baseline FEV_1 between 40% and 90% pred that approximated the original trial results [4–7].

Interestingly, the mean acute improvement of FEV_1 in participants with a baseline FEV_1 <40% pred was not significantly different from the group with a pre-modulator FEV_1 40%–90% pred. Moreover, the improvement of FEV_1 decline was even higher in those with FEV_1 <40% pred before CFTR modulator initiation. Similar short-term improvements in pwCF and severe lung disease were already reported in subgroup analyses of clinical trials and in several case series [11], but the long-term benefits in this subgroup have not yet been demonstrated before.

In addition, long-term changes in BMI and BMI Z-score in this study were moderate compared to previous trials [6,7], and despite the acute decrease in the duration of *i.v.* antibiotic use in the first year after modulator initiation, the mean duration of *i.v.* antibiotic treatment continued to increase again in the subsequent years.

Taken together, the results of this study emphasise that translation of clinical trial results into daily clinical practice can be difficult, especially in chronic diseases like CF. Most of the discrepancies are probably explained by the different populations, design and settings of traditional trials compared to observational real-world

studies. This could be related to the relatively short follow-up of RCTs, as well as to the stringent selection criteria which usually exclude people with, for example, severe or limited lung disease (FEV_1 <40% pred and >90% pred) or people with CF-related comorbidities such as diabetes and liver disease. In addition, clinical trial conditions regarding co-medication and treatment adherence are strictly controlled, whereas temporary or permanent treatment discontinuation is more likely to occur in practice [8]. Real-world studies with a long-term follow-up are therefore important to provide additional post-approval data of the impact and sustainability of treatments on the entire heterogeneous population [12].

So far, seven studies have been published that assessed the effectiveness of LUM/IVA in a real-world setting [13–19]. Most of these studies were conducted in small populations, examining different subgroups and outcomes with a follow-up period of 1 year after LUM/IVA initiation and a limited observation period, not exceeding 845 patient-years.

The present study substantially contributes to the existing real-world evidence, because the follow-up period covered on average 7 years before CFTR modulator treatment and up to 3 years after modulator initiation. Moreover, this study included 3844 patient-years of observation of a relatively large and heterogeneous population of F508del-homozygous pwCF aged 12 years and older at different disease stages, which reflects daily clinical practice. In addition, we adjusted for the confounding effect of age, which is known to be associated with rate of lung function decline [20].

Overall, our results were consistent with previous studies that suggested real-world effectiveness to be less pronounced compared to the initial trials. Most studies reported a moderate change from baseline FEV_1 % pred [13,14] and a moderate change in FEV_1 % pred decline after 1 year [16,17] or 2 years [18] of follow-up. The discrepancy with a different recently published study that focused on predictors of long-term clinical outcomes using encounter-based FEV1 measurements [19] might be explained by the inclusion of annual best FEV1 measurements in the NCFR. Annual best measurements may provide a better estimation of long-term trends, as this reduces the impact of measurement variability over time compared to multiple repeated measurements. Given the strong (nonlinear) association of lung function decline with age [20,21], trends were adjusted for age at baseline in this study. The short- and long-term improvement of BMI and nutritional status could be interpreted as modest and was more profound in adolescents [13,14,18]. The use of the different reference values for adults and adolescents limits a direct comparison of BMI and BMI Z-score trends between age groups, which has also not

been assessed in other real-world studies. Nevertheless, similar differences were reported in the PROGRESS trial, showing an increasing BMI trend in treated pwCF and matched registry controls, whereas BMI Z-score and weight-for-age trend improved after LUM/IVA initiation compared to a decline in matched registry controls [6]. Moreover, LUM/IVA and TEZ/ IVA might induce a short-term improvement of pulmonary exacerbations [13,14] and reduce the use of *i.v.* antibiotics in the first year after treatment initiation in pwCF above 12 years of age, but this improvement was not sustained in the subsequent years [18,19]. This could indicate that the benefit of dual CFTR modulators on severe pulmonary exacerbations diminishes in the long-term, but it could also be related to a decreasing long-term adherence to modulators or to a reduced prescription or adherence to other co-medication such as dornase α, hypertonic saline and inhaled antibiotics in a real-life setting.

The contrast between short- and long-term changes in this study also illustrates that traditional short-term clinical end-points such as FEV_1 % pred might not always be the best measures to capture treatment benefits, especially when effect sizes are limited, populations are heterogeneous and sample sizes are small, which frequently occurs in rare diseases such as CF. Long-term trials or observational real-world studies might partially overcome this problem because they could reveal an inhibition of disease progression, but alternative approaches will be needed since long-term studies are not always feasible and require sufficient short-term evidence first.

An important limitation of this study was the relatively large proportion of missing data in *i.v.* antibiotic treatment duration, which was not consistently collected in the NCFR throughout the entire study period, particularly in the years before CFTR modulator initiation (2010–2014). Although we used appropriate statistical models to adjust for missing data, we cannot rule out that this might have influenced the results. Even though we did adjust for the most important confounders age and sex, we were not able to include data regarding either treatment discontinuation and side-effects or concomitant treatments such as hydrators, dornase α, azithromycin or other inhaled or oral antibiotics, which might have respectively underestimated or overestimated the reported effectiveness. Due to the transition from LUM/IVA to TEZ/IVA during the observation period, this study provides combined results about the effectiveness of both dual CFTR modulators. Based on the additional subgroup analyses that compared the groups who did and did not switch to TEZ/IVA, the influence of transition was considered as limited.

In conclusion, this real-world study showed that long-term FEV_1 decline improved up to 3 years after the introduction of LUM/IVA and TEZ/IVA, which was also

observed for BMI Z-score in children, but not for BMI in adults. Intravenous antibiotic treatment duration was reduced in the first year after modulator initiation, but this duration increased in the subsequent years. Compared to the efficacy reported in previous clinical trials, real-world effectiveness of the dual CFTR modulators is less pronounced and varies considerably between pwCF and different baseline FEV_1 levels.

Acknowledgements
We thank all people with cystic fibrosis who consented to the collection of their data by the Dutch Cystic Fibrosis Registry (NCFR) and the use of these data for research. We also thank all collaborating Dutch CF centers for their effort to provide the data to the NCFR, in particular the members of the NCFR Steering Group.

Dutch Cystic Fibrosis Registry (NCFR) Steering Group: J. Altenburg, Amsterdam University Medical Centers; S.W.J. Terheggen-Lagro, Emma Children's Hospital, Amsterdam University Medical Centers; H.G.M. Heijerman, University Medical Center Utrecht; K.M. de Winter-de Groot, Wilhelmina Children's Hospital, University Medical Center Utrecht; M. Bakker, Erasmus University Medical Center, Rotterdam; R.A.S. Hoek, Erasmus University Medical Center, Rotterdam; H.M. Janssens, Sophia Children's Hospital, Erasmus University Medical Center, Rotterdam; R. van der Meer, Haga Teaching Hospital, The Hague; M. Nuijsink, Juliana Children's Hospital, Haga Teaching Hospital, The Hague; H. van der Vaart, University Medical Center Groningen; G.H. Koppelman, Beatrix Children's Hospital, University Medical Center Groningen; L.H. Conemans, Maastricht University Medical Center+, Maastricht; M.A.G.E. Bannier, Maastricht University Medical Center+, Maastricht, P.F.J.M. Merkus, Amalia Children's Hospital, Radboud University Medical Center, Nijmegen; and J.J. Noordhoek, Dutch Cystic Fibrosis Foundation; all in The Netherlands.

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Conflicts of interest

D. Muilwijk has nothing to disclose. D.D. Zomer-van Ommen has nothing to disclose. V.A.M. Gulmans has nothing to disclose. M.J.C. Eijkemans has nothing to disclose. C.K. van der Ent reports grants from Vertex, Eloxx, Proteostasis, Galapagos NV, ProQR, Gilead, TEVA, GSK and Nutricia (money to institution), outside the submitted work.

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SUPPLEMENTARY **Materials and methods.**

Statistical model specification

ppFEV1

A linear mixed effects model was used to assess longitudinal trends in ppFEV1 before and after CFTR modulator initiation. The model included a random intercept per subject and random slopes for time, CFTR modulator treatment status and the interaction between time and CFTR modulator treatment status, using an unstructured covariance matrix. As fixed effects we included time, CFTR modulator treatment status and the interaction between time and CFTR modulator treatment status in the unadjusted model. The fixed effect for time represented ppFEV1 decline in the years before CFTR modulator use and the interaction of time : CFTR modulator treatment reflected ppFEV1 decline after CFTR modulator initiation. Age at baseline (centered to median) and sex were considered as (potential) confounders, as ppFEV1 decline is associated with age [1,2] and could be different between males and females [3]. We used stepwise forward selection to test these variables as two-way interaction terms with time and as three-way interactions with time : CFTR modulator treatment. The interaction terms that significantly improved model fit, indicating a significant association, were included in the final adjusted model.

For the subgroup analyses, the same linear mixed effects models were built, including additional interaction terms of time, CFTR modulator treatment and time : CFTR modulator treatment with 1) baseline ppFEV1 category (<40%, between 40-90% and ≥90%); 2) age category (adults > 18 years and adolescents 12-18 years); 3) CFTR modulator transition to TEZ/IVA or continuation of LUM/IVA; and 4) female or male sex.

BMI and BMI Z-score

Following the same approach, the analyses of BMI and BMI Z-score were performed in data subsets including measurements at an age above and below 19 years, respectively, based on WHO growth reference guidelines for normalization of BMI Z-score. These linear mixed effects models included a random intercept per subject and random slopes for time and the interaction between time and CFTR modulator treatment status. Time, CFTR modulator treatment status and the interaction between time and CFTR modulator treatment status were added as fixed effects in the unadjusted models. In addition, main effects and statistically significant interaction terms with sex and age at baseline (centered to median) were added to the adjusted models. As the data subsets for BMI and BMI Z-score were already divided by age category and were too small to allow for subgroup analysis with

baseline ppFEV1 categories, we only conducted additional subgroup analysis for the transition or continuation of CFTR modulator type and for sex.

IV antibiotic treatment duration

Changes in the annual duration of IV antibiotic treatment were analyzed with a negative binomial mixed effects model. A random intercept per subject was included, assuming an unstructured covariance matrix. As fixed effects in the unadjusted model, we included time, CFTR modulator treatment status and the interaction between time and CFTR modulator treatment status, which reflected the change in duration of IV antibiotic treatment in the years after CFTR modulator initiation. Finally, main effects and statistically significant interaction terms with sex and age at baseline (centered to median) were added to the adjusted models. Similar to ppFEV1, additional subgroup analyses were performed using negative binomial mixed effects models with same structure as the main model.

Supplementary table S1a. Bayesian linear mixed effects model estimates of ppFEV1. Comparison of effects in participants with baseline FEV1pp <40%, between 40-90% and ≥90% (n=401, Years of observation=3844).

	Unadjusted coefficient	95% CI	P-value	Adjusted coefficient#	95% CI	P-value
Intercept	67.46	65.91 – 69.00	<0.001	68.74	67.05 – 70.41	<0.001
ppFEV1 40-90%	Reference			Reference		
ppFEV1 <40%	-34.30	-37.87 – -30.75	<0.001*	-29.46	-32.74 – -26.19	<0.001*
ppFEV1 ≥90%	29.03	26.20 – 31.86	<0.001*	25.13	22.49 – 27.76	<0.001*
Time	-1.59	-1.82 – -1.36	<0.001*	-1.62	-1.84 – -1.41	<0.001*
Time : ppFEV1 40-90%	Reference			Reference		
Time : ppFEV1 <40%	-1.12	-2.50 – 0.26	0.112	-2.24	-3.58 – -0.90	0.001*
Time : ppFEV1 ≥90%	2.25	1.49 – 3.01	<0.001*	2.87	2.13 – 3.62	<0.001*
CFTR modulator	2.60	1.42 – 3.78	<0.001*	2.59	1.40 – 3.78	<0.001*
CFTR modulator : ppFEV1 40%-90%	Reference			Reference		
CFTR modulator : ppFEV1 <40%	-1.30	-4.30 – 1.69	0.395	-1.24	-4.25 – 1.78	0.420
CFTR modulator : ppFEV1 ≥90%	-4.12	-6.41 – -1.82	0.002*	-4.07	-6.36 – -1.77	<0.001*
Time : CFTR modulator	0.75	0.05 – 1.43	0.039*	0.81	0.11– 1.50	0.026*
Time : CFTR modulator : ppFEV1 40-90%	Reference			Reference		
Time : CFTR modulator : ppFEV1 <40%	1.50	0.10 – 2.92	0.035*	1.40	-0.0001 – 2.82	0.050*
Time : CFTR modulator : ppFEV1 ≥90%	-0.60	-1.97 – 0.7	0.389	-0.63	-2.01 – 0.76	0.368

Definitions and abbreviations: percent predicted forced expiratory volume in 1 s (ppFEV1), lumacaftor/ivacaftor (LUM/IVA), tezacaftor/ivacaftor (TEZ/IVA), Cystic Fibrosis Transmembrane conductance Regulator (CFTR). Time in years. # Adjusted for sex, age at baseline and the interaction effect between age at baseline with time. * Significance level < 0.05.

Supplementary table S1b. Bayesian linear mixed effects model estimates of ppFEV1. Comparison of effects in participants who transitioned to TEZ/IVA and participants who continued LUM/IVA treatment (n=401, Years of observation=3844).

	Unadjusted coefficient	95% CI	P-value	Adjusted coefficient#	95% CI	P-value
Intercept	66.33	63.16 – 69.48	<0.001	70.20	67.25 – 73.14	<0.001
Continuation LUM/IVA	Reference			Reference		
Transition TEZ/IVA	5.22	0.98 – 9.48	0.017*	1.64	-1.95 – 5.21	0.365
Time	-1.22	-1.51 – -0.94	<0.001*	-1.28	-1.56 – -1.00	<0.001*
Time : continuation LUM/IVA	Reference			Reference		
Time : transition TEZ/IVA	-0.28	-0.74 – 0.18	0.234	-0.17	-0.63 – 0.29	0.462
CFTR modulator	1.81	0.41 – 3.21	0.011*	1.79	0.37 – 3.21	0.014*
CFTR modulator : continuation LUM/IVA	Reference			Reference		
CFTR modulator : transition TEZ/IVA	-0.56	-2.47 – 1.34	0.562	-0.52	-2.46 – 1.39	0.593
Time : CFTR Modulator	1.01	0.22 – 1.80	0.016*	1.03	0.22 – 1.83	0.013*
Time : CFTR modulator : continuation LUM/IVA	Reference			Reference		
Time : CFTR modulator : transition TEZ/IVA	-0.26	-1.30 – 0.79	0.629	-0.27	-1.34 – 0.80	0.612

Adjusted for sex, age at baseline and the interaction effect between age at baseline and time. * Significance level < 0.05.

Supplementary table S1c. Bayesian linear mixed effects model estimates of ppFEV1. Comparison of effects in females and males (n=401, Years of observation=3844).

	Unadjusted coefficient	95% CI	P-value	Adjusted coefficient#	95% CI	P-value
Intercept	68.12	65.20 – 71.06	<0.001	70.89	68.39 – 73.38	<0.001
Male sex	Reference			Reference		
Female sex	2.30	-2.04 – 6.62	0.300	0.19	-3.37 – 3.75	0.918
Time	-1.35	-1.61 – -1.09	<0.001*	-1.38	-1.64 – -1.13	<0.001*
Time : male sex	Reference			Reference		
Time : female sex	0.01	-0.50 – 0.51	0.973	0.08	-0.42 – 0.58	0.762
CFTR modulator	1.43	0.15 – 2.70	0.028*	1.38	0.11 – 2.64	0.033*
CFTR modulator : male sex	Reference			Reference		
CFTR modulator : female sex	0.26	-1.67 – 2.19	0.797	0.32	-1.63 – 2.26	0.749
Time : CFTR Modulator	0.55	0.23 – 1.62	0.013*	0.97	0.27 – 1.65	0.011*
Time : CFTR modulator : male sex	Reference			Reference		
Time : CFTR modulator : female sex	-0.16	-1.24 – 0.91	0.767	-0.22	-1.29 – 0.86	0.685

Adjusted for age at baseline and the interaction effect between age at baseline and time. * Significance level < 0.05.

Supplementary table S1d. Bayesian linear mixed effects model estimates of ppFEV1. Comparison of effects in adults >18 years and adolescents of 12-18 years (n=401, Years of observation=3844).

	Unadjusted coefficient	95% CI	P-value	Adjusted coefficient#	95% CI	P-value
Intercept	86.22	82.66 – 89.77	<0.001	85.57	81.69 – 89.48	<0.001
Adolescents	Reference			Reference		
Adults	-24.08	-28.19 – -20.01	<0.001*	-24.01	-28.14 – -19.91	<0.001*
Time	-1.55	-1.91 – -1.18	<0.001*	-1.11	-1.91 – -1.18	<0.001*
Time : adolescents	Reference			Reference		
Time : adults	0.29	-0.18 – 0.75	0.215	0.29	-0.17 – 0.76	0.215
CFTR modulator	0.15	-1.60 – 1.91	0.881	0.14	-1.61 – 1.88	0.893
CFTR modulator : adolescents	Reference			Reference		
CFTR modulator : adults	1.95	-0.15 – 4.01	0.070	1.95	-0.14 – 4.04	0.067
Time : CFTR Modulator	0.48	-0.47 – 1.49	0.314	0.50	-0.46 – 1.45	0.289
Time : CFTR modulator : adolescents	Reference			Reference		
Time : CFTR modulator : adults	0.57	-0.59 – 1.72	0.334	0.55	-0.58 – 1.70	0.342

Adjusted for sex. * Significance level < 0.05.

Supplementary table S2a. Bayesian linear mixed effects model estimates of BMI in adults ≥ 19 years. Subgroup analysis in participants with baseline ppFEV1 40-90% (n=214, Years of observation=1564)

	Unadjusted coefficient	95% CI	P-value	Adjusted coefficient#	95% CI	P-value
Intercept	21.35	21.03 – 21.66	<0.001	21.27	20.85 – 21.69	<0.001
Time	0.05	0.01 – 0.09	0.021*	0.06	0.02 – 0.11	0.008*
CFTR modulator	0.12	-0.05 – 0.30	0.175	0.14	-0.04 – 0.32	0.121
Time : CFTR Modulator	0.08	-0.04 – 0.20	0.212	0.13	-0.004 – 0.26	0.058

Definitions and abbreviations: body mass index (BMI) in kg/m^2. # Adjusted for sex, age at baseline and the interaction effect between age at baseline and time and between age at baseline, time and CFTR modulator treatment. * Significance level < 0.05.

Supplementary table S2b. Bayesian linear mixed effects model estimates of BMI in adults ≥ 19 years. Comparison of effects in participants who transitioned to TEZ/IVA and participants who continued LUM/IVA treatment (n=312, Years of observation=2317).

	Unadjusted coefficient	95% CI	P-value	Adjusted coefficient#	95% CI	P-value
Intercept	21.46	21.10 – 21.82	<0.001	21.40	21.00 – 21.81	<0.001
Continuation LUM/IVA	Reference			Reference		
Transition TEZ/IVA	-0.14	-0.66 – 0.38	0.604	-0.08	-0.60 – 0.44	0.765
Time	0.05	0.004 – 0.09	0.033*	0.07	0.02 – 0.11	0.005*
Time : continuation LUM/IVA	Reference			Reference		
Time : transition TEZ/IVA	0.03	-0.06 – 0.12	0.572	0.04	-0.05 – 0.13	0.350
CFTR modulator	0.28	0.01 – 0.44	0.038*	0.22	0.001 – 0.44	0.049*
CFTR modulator : continuation LUM/IVA	Reference			Reference		
CFTR modulator : transition TEZ/IVA	-0.20	-0.52 – 0.12	0.219	-0.20	-0.54 – 0.12	0.227
Time : CFTR Modulator	0.10	-0.04 – 0.25	0.147	0.14	-0.003 – 0.28	0.055
Time : CFTR modulator : continuation LUM/IVA	Reference			Reference		
Time : CFTR modulator : transition TEZ/IVA	-0.08	-0.28 – 0.12	0.434	-0.08	-0.27 – 0.10	0.387

Adjusted for the main effects of sex, age at baseline, the interaction effect of age at baseline with time and the interaction effect of age at baseline with time and CFTR modulator treatment. * Significance level < 0.05.

Supplementary table S2c. Bayesian linear mixed effects model of BMI in adults ≥ 19 years. Comparison of effect estimates in females and males (n=312, Years of observation=2317).

	Unadjusted coefficient	95% CI	P-value	Adjusted coefficient#	95% CI	P-value
Intercept	21.69	21.35 – 22.03	<0.001	21.32	20.95 – 21.69	<0.001
Male sex	Reference			Reference		
Female sex	-0.77	-1.29 – -0.25	0.004*	-0.71	-1.22 – -0.19	0.007*
Time	0.07	0.02 – 0.11	0.002*	0.09	0.05 – 0.14	<0.001*
Time : male sex	Reference			Reference		
Time : female sex	-0.04	-0.14 – 0.06	0.443	-0.04	-0.14 – 0.05	0.360
CFTR modulator	0.26	0.06 – 0.48	0.012*	0.26	0.05 – 0.48	0.016*
CFTR modulator : male sex	Reference			Reference		
CFTR modulator : female sex	-0.29	-0.62 – 0.03	0.078	-0.30	-0.63 – 0.03	0.078
Time : CFTR Modulator	0.03	-0.10 – 0.17	0.653	0.07	-0.07 – 0.20	0.330
Time : CFTR modulator : male sex	Reference			Reference		
Time : CFTR modulator : female sex	0.08	-0.12 – 0.29	0.432	0.08	-0.11 – 0.27	0.406

Adjusted for the main effects of sex, age at baseline, the interaction effect of age at baseline with time and the interaction effect of age at baseline with time and CFTR modulator treatment. * Significance level < 0.05.

Supplementary table S3a. Bayesian linear mixed effects model estimates of BMI Z-score in children < 19 years. Subgroup analysis in participants with baseline ppFEV1 40-90% (n=147, Years of observation=941)

	Unadjusted coefficient	95% CI	P-value	Adjusted coefficient#	95% CI	P-value
Intercept	-0.71	-0.87 – -0.55	<0.001	-0.82	-1.09 – -0.54	<0.001
Time	-0.07	-0.10 – -0.05	<0.001*	-0.08	-0.12 – -0.05	<0.001*
CFTR modulator	-0.07	-0.28 – 0.14	0.502	0.01	-0.21 – 0.22	0.924
Time : CFTR Modulator	0.08	-0.04 – 0.19	0.181	0.09	-0.02 – 0.20	0.113

Definitions and abbreviations: BMI Z-score was normalized for age and sex and according to the WHO growth reference standard. # Adjusted for the main effects of sex, age at baseline, the interaction effect of sex with time and the interaction effect of age at baseline with time. * Significance level p<0.05.

Supplementary table S3b. Bayesian linear mixed effects model estimates of BMI Z-score in children < 19 years. Comparison of effects in participants who transitioned to TEZ/IVA and participants who continued LUM/IVA treatment (n=225, Years of observation=1552).

	Unadjusted coefficient	95% CI	P-value	Adjusted coefficient#	95% CI	P-value
Intercept	-0.71	-0.89 – -0.53	<0.001	-0.87	-1.11 – -0.64	<0.001
Continuation LUM/IVA	Reference			Reference		
Transition TEZ/IVA	0.19	-0.001 – 0.38	0.051	0.12	-0.09 – 0.32	0.253
Time	-0.05	-0.08 – -0.01	0.011*	-0.07	-0.10 – -0.03	<0.001*
Time : continuation LUM/IVA	Reference			Reference		
Time : transition TEZ/IVA	-0.04	-0.08 – 0.005	0.083	-0.02	-0.07 – 0.02	0.283
CFTR modulator	0.16	-0.11 – 0.44	0.242	0.14	-0.17 – 0.44	0.375
CFTR modulator : continuation LUM/IVA	Reference			Reference		
CFTR modulator : transition TEZ/IVA	-0.17	-0.47 – 0.13	0.271	-0.11	-0.45 – 0.24	0.520
Time : CFTR Modulator	0.02	-0.21 – 0.26	0.862	0.11	-0.07 – 0.30	0.228
Time : CFTR modulator : continuation LUM/IVA	Reference			Reference		
Time : CFTR modulator : transition TEZ/IVA	0.11	-0.15 – 0.37	0.398	0.03	-0.18 – 0.23	0.786

Adjusted for the main effects of sex, age at baseline, the interaction effect of sex with time and the interaction effect of age at baseline with time. * Significance level p<0.05.

Supplementary table S3c. Bayesian linear mixed effects model estimates of BMI Z-score in children < 19 years. Comparison of effects in females and males (n=225, Years of observation=1552).

	Unadjusted coefficient	95% CI	P-value	Adjusted coefficient#	95% CI	P-value
Intercept	-0.58	-0.73 – -0.42	<0.001	-0.83	-1.05 – -0.60	<0.001
Male sex	Reference			Reference		
Female sex	-0.05	-0.24 – 0.15	0.639	-0.05	-0.24 – 0.14	0.594
Time	-0.07	-0.10 – -0.04	<0.001*	-0.07	-0.09 – -0.04	<0.001*
Time : male sex	Reference			Reference		
Time : female sex	0.002	-0.04 – 0.05	0.919	0.004	-0.04 – 0.05	0.853
CFTR modulator	-0.14	-0.29 – 0.02	0.086	-0.11	-0.29 – 0.08	0.270
CFTR modulator : male sex	Reference			Reference		
CFTR modulator : female sex	0.33	0.09 – 0.56	0.007*	0.33	0.06 – 0.61	0.018*
Time : CFTR Modulator	0.10	-0.04 – 0.23	0.161	0.12	0.02 – 0.23	0.023*
Time : CFTR modulator : male sex	Reference			Reference		
Time : CFTR modulator : female sex	0.01	-0.19 – 0.21	0.898	0.03	-0.13 – 0.19	0.717

Adjusted for the main effects of sex, age at baseline, the interaction effect of sex with time and the interaction effect of age at baseline with time. * Significance level p<0.05.

Supplementary table S4a. Negative binomial mixed effects model estimates of the duration of IV antibiotic treatment. Comparison of effects in participants with baseline FEV1pp <40%, between 40-90% and >=90% (n=361, Years of observation=2827).

	Unadjusted coefficient	IRR	95% CI (IRR)	P-value	Adjusted coefficient#	IRR	95% CI (IRR)	P-value
Intercept	2.01	7.49	4.82 – 11.63	<0.001	1.81	6.16	3.81 – 9.97	<0.001
ppFEV1 40-90%	Reference	Reference			Reference	Reference		
ppFEV1 <40%	1.08	2.96	1.04 – 8.44	0.043*	1.10	3.02	1.04 – 8.77	0.042*
ppFEV1 >=90%	-1.48	0.23	0.09 – 0.55	0.001*	-1.51	0.22		<0.001*
Time	0.09	1.09	0.99 – 1.20	0.080	0.09	1.09	0.99 – 1.20	0.079
Time : ppFEV1 40-90%	Reference	Reference			Reference	Reference		
Time : ppFEV1 <40%	0.02	1.02	0.78 – 1.34	0.879	0.03	1.03	0.78 – 1.35	0.851
Time : ppFEV1 >=90%	0.31	1.36	1.11 – 1.66	0.003*	0.31	1.36	1.11 – 1.66	0.003*
CFTR modulator	-1.19	0.30	0.19 – 0.48	<0.001*	-1.20	0.30	0.19 – 0.47	<0.001*
CFTR modulator : ppFEV1 40%-90%	Reference	Reference			Reference	Reference		
CFTR modulator : ppFEV1 <40%	0.01	1.01	0.34 – 3.01	0.990	0.03	1.03	0.34 – 3.14	0.954
CFTR modulator : ppFEV1 >=90%	-0.08	0.92	0.36 – 2.34	0.866	-0.07	0.93	0.36 – 2.39	0.881
Time : CFTR modulator	0.25	1.29	0.97 – 1.70	0.077	0.26	1.30	0.98 – 1.72	0.071
Time : CFTR modulator : ppFEV1 40-90%	Reference	Reference			Reference	Reference		
Time : CFTR modulator : ppFEV1 <40%	0.12	1.12	0.58 – 2.18	0.730	0.10	1.10	0.56 – 2.16	0.774
Time : CFTR modulator : ppFEV1 >=90%	-1.04	0.35	0.17 – 0.73	0.005*	-1.03	0.36	0.17 – 0.75	0.006*

Definitions and abbreviations: intravenous (IV). Coefficients are on the log-scale. Incidence rate ratios (IRR) are transformed back to the original scale. # Adjusted for age at baseline and sex. * Significance level < 0.05.

Supplementary table S4b. Negative binomial mixed effects model estimates of the duration of IV antibiotic treatment. Comparison of effects in participants who transitioned to TEZ/IVA and participants who continued LUM/IVA treatment (n=364, Years of observation=2848).

	Unadjusted coefficient	IRR	95% CI (IRR)	P-value	Adjusted coefficient#	IRR	95% CI (IRR)	P-value
Intercept	1.81	6.13	3.43 – 10.97	<0.001	1.54	4.64	2.53 – 8.54	<0.001
Continuation LUM/IVA	Reference	Reference			Reference	Reference		
Transition TEZ/IVA	-0.04	0.96	0.45 – 2.03	0.914	-0.03	0.97	0.46 – 2.07	0.944
Time	0.21	1.23	1.08 – 1.41	0.002*	0.21	1.23	1.08 – 1.41	0.002*
Time : continuation LUM/IVA	Reference	Reference			Reference	Reference		
Time : transition TEZ/IVA	-0.09	0.91	0.77 – 1.08	0.284	-0.09	0.91	0.77 – 1.08	0.277
CFTR modulator	-1.47	0.23	0.13 – 0.41	<0.001*	-1.49	0.23	0.13 – 0.40	<0.001*
CFTR modulator : continuation LUM/IVA	Reference	Reference			Reference	Reference		
CFTR modulator : transition TEZ/IVA	0.31	1.37	0.64 – 2.90	0.415	0.34	1.40	0.66 – 2.97	0.375
Time : CFTR Modulator	0.16	1.18	0.81 – 1.71	0.391	0.17	1.19	0.82 – 1.73	0.361
Time : CFTR modulator : continuation LUM/IVA	Reference	Reference			Reference	Reference		
Time : CFTR modulator : transition TEZ/IVA	-0.01	0.99	0.61 – 1.60	0.969	-0.02	0.98	0.61 – 1.58	0.937

Adjusted for age at baseline and sex. * Significance level < 0.05.

Supplementary table S4c. Negative binomial mixed effects model estimates of the duration of IV antibiotic treatment. Comparison of effects in females and males (n=364, Years of observation=2848).

	Unadjusted coefficient	IRR	95% CI (IRR)	P-value	Adjusted coefficient#	IRR	95% CI (IRR)	P-value
Intercept	1.57	4.81	2.85 – 8.12	<0.001	1.51	4.53	2.68 – 7.66	<0.001
Male sex	Reference	Reference			Reference	Reference		
Female sex	0.46	1.58	0.75 – 3.31	0.226	0.50	1.65	0.79 – 3.45	0.186
Time	0.15	1.16	1.03 – 1.30	0.014*	0.15	1.16	1.03 – 1.30	0.015*
Time : male sex	Reference	Reference			Reference	Reference		
Time : female sex	0.001	1.00	0.85 – 1.78	0.984	0.002	1.00	0.85 – 1.78	0.977
CFTR modulator	-1.46	0.23	0.14 – 0.39	<0.001*	-1.45	0.23	0.14 – 0.39	<0.001*
CFTR modulator : male sex	Reference	Reference			Reference	Reference		
CFTR modulator : female sex	0.38	1.47	0.70 – 3.08	0.312	0.37	1.45	0.69 – 3.05	0.325
Time : CFTR Modulator	0.28	1.32	0.96 – 1.80	0.084	0.28	1.32	0.97 – 1.81	0.081
Time : CFTR modulator : male sex	Reference	Reference			Reference	Reference		
Time : CFTR modulator : female sex	-0.24	0.78	0.49 – 1.26	0.313	-0.25	0.78	0.49 – 1.25	0.297

Adjusted for age at baseline. * Significance level < 0.05.

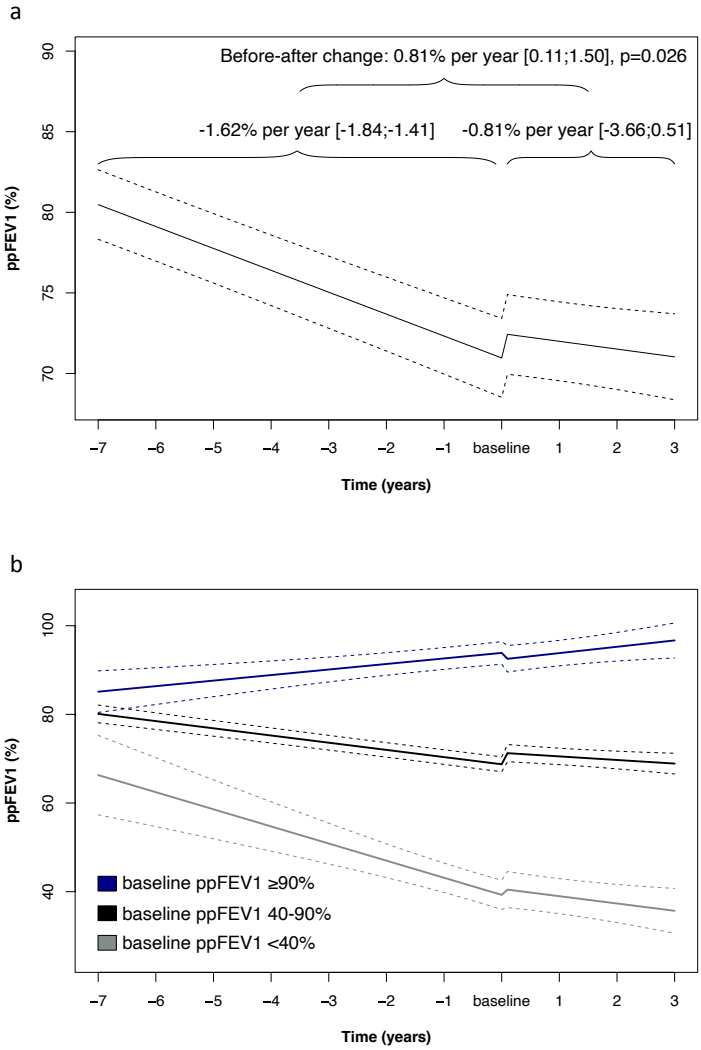
Supplementary table S4d. Negative binomial mixed effects model estimates of the duration of IV antibiotic treatment. Comparison of effects in adults >18 years and adolescents of 12-18 years (n=364, Years of observation=2848).

	Unadjusted coefficient	IRR	95% CI (IRR)	P-value	Adjusted coefficient#	IRR	95% CI (IRR)	P-value
Intercept	1.49	4.45	2.21 – 8.95	<0.001	2.25	9.49	4.91 – 18.33	<0.001
Adolescents	Reference	Reference			Reference	Reference		
Adults	0.38	1.46	0.65 – 3.30	0.361	0.36	1.44	0.68 – 3.03	0.341
Time	0.24	1.28	1.10 – 1.48	0.001*	0.22	1.25	1.07 – 1.45	0.005*
Time : adolescents	Reference	Reference			Reference	Reference		
Time : adults	-0.14	0.87	0.73 – 1.04	0.122	-0.10	0.90	0.75 – 1.09	0.284
CFTR modulator	-1.61	0.20	0.10 – 0.40	<0.001*	-1.08	0.34	0.16 – 0.71	0.004*
CFTR modulator : adolescents	Reference	Reference			Reference	Reference		
CFTR modulator : adults	0.48	1.62	0.71 – 3.71	0.252	-0.03	0.97	0.40 – 2.35	0.948
Time : CFTR Modulator	0.04	1.04	0.66 – 1.64	0.855	0.10	1.11	0.68 – 1.79	0.685
Time : CFTR modulator : adolescents	Reference	Reference			Reference	Reference		
Time : CFTR modulator : adults	0.17	1.19	0.70 – 2.02	0.516	0.16	1.17	0.67 – 2.06	0.583

Adjusted for sex. * Significance level < 0.05.

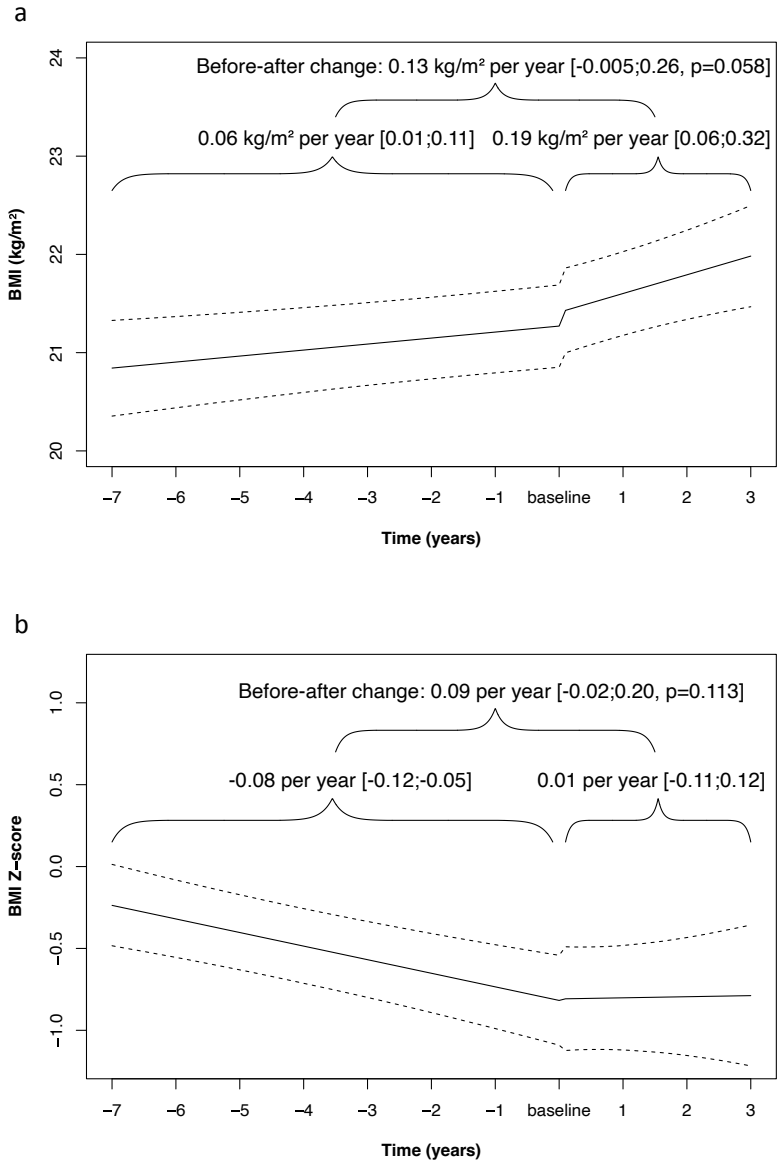
SUPPLEMENTARY **Figures.**

Supplementary figure S1. Comparison of longitudinal ppFEV1 trends before and after CFTR modulator initiation in subgroups with baseline ppFEV1 <40%, between 40-90% and ≥90%



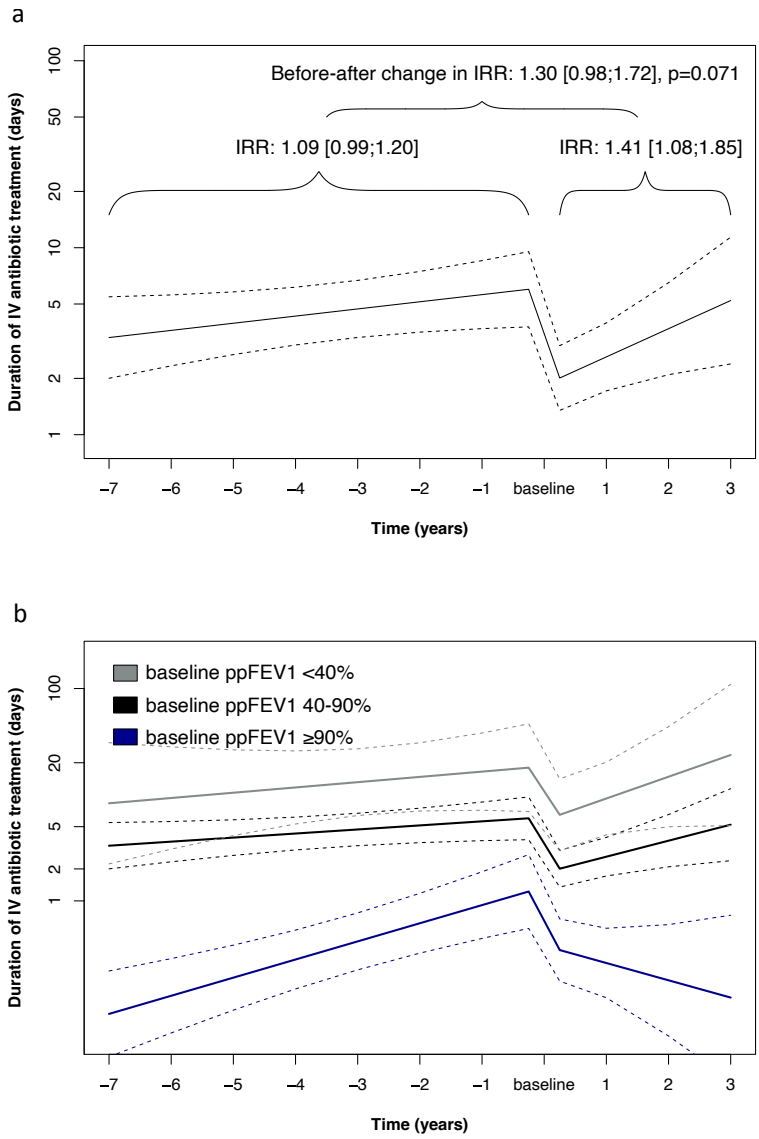
Time ranges from -7 years before to +3 years after CFTR modulator initiation, with time=0 (baseline) defined by the start date of CFTR modulator treatment. Dashed lines represent 95% confidence intervals, which are also shown between square brackets. Panel 1a: The impact of CFTR modulator use in the subgroup with baseline ppFEV1 40-90% was demonstrated by an acute change from baseline ppFEV1 of 2.59% (95% CI: 1.40 – 3.78%, p<0.001) in addition to an improvement in ppFEV1 decline of 0.81% per year, 95% CI: 0.11 – 1.50%, p=0.026); **Supplementary table S1a**) that was comparable to the main analysis. Panel 1b: Compared to the group with baseline ppFEV1 40-90% (black lines), the average estimated change in ppFEV1 decline after CFTR modulator initiation was on average even 1.40% per year higher (95% CI -0.0001 - 2.82%, p=0.050; **Supplementary table S1a**) in the group with baseline ppFEV1 <40% (grey lines). In the group with baseline ppFEV1 ≥90% (dark blue lines), a longitudinal decline in ppFEV1 was not observed.

Supplementary figure S2. Comparison of longitudinal BMI and BMI Z-score trends before and after CFTR modulator initiation in subgroup with baseline ppFEV1 between 40-90%



Time ranges from -7 years before to +3 years after CFTR modulator initiation, with time=0 (baseline) defined by the start date of CFTR modulator treatment. Dashed lines represent 95% confidence intervals, which are also reported between square brackets. Panel 2a: In adults ≥19 years, BMI trend before and after CFTR modulator initiation in this subgroup was comparable to the observed overall trends, with a change in annual BMI of 0.13 (95% CI: -0.04 – 0.32, p=0.058) after CFTR modulator initiation (**Supplementary table S2a**). Panel 2b: Trends of BMI Z-score in children <19 years were similar to the entire population, although the longitudinal change after CFTR modulator initiation was slightly smaller compared to the entire cohort (change: 0.09 per year, 95% CI: -0.02 – 0.20, p=0.113; **Supplementary table S3a**).

Supplementary figure S3. Comparison of longitudinal trends in IV antibiotic treatment duration before and after CFTR modulator initiation in subgroups with baseline ppFEV1 <40%, between 40-90% and ≥90%



Time ranges from -7 years before to +3 years after CFTR modulator initiation, with time=0 (baseline) defined by the start date of CFTR-modulator treatment. Dashed lines represent 95% confidence intervals, which are also reported between square brackets. Panel 3a: trends in the average annual duration of IV antibiotic treatment (in days) were comparable to the overall population, although the average duration of received IV antibiotics in the last year preceding CFTR modulator initiation was slightly higher (6.16 days, 95% CI: 5.32 – 15.38 days; **Supplementary table S4a**). Panel 3b: Compared to the group with baseline ppFEV1 40-90% (black lines), average trends of participants with a baseline ppFEV1 <40% (grey lines) were comparable to participants with baseline ppFEV1 40-90%, but the average IV antibiotic treatment duration in participants with a baseline ppFEV1 ≥90% (dark blue lines) was considerably lower and did not increase after CFTR modulator initiation (**Supplementary table S4a**).

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CHAPTER 5

Prediction of real-world long-term outcomes of people with CF homozygous for the F508del mutation treated with CFTR modulators

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Journal of Personalized Medicine 2021, Dec 16;11(12):1376

ABSTRACT

Introduction

The clinical response to cystic fibrosis transmembrane conductance regulator (CFTR) modulators is variable within people with cystic fibrosis (pwCF) homozygous for the F508del mutation. The prediction of clinical effect in individual patients would be useful to target therapy to those who would benefit from it.

Methods

A multicenter observational cohort study was conducted including 97 pwCF (F508del/F508del), who started lumacaftor/ivacaftor (LUM/IVA) treatment before June 2018. In order to assess the associations of individual *in vivo* and *in vitro* biomarkers with clinical outcomes, we collected clinical data regarding sex, age, and sweat chloride concentration (SwCl) at baseline and after six months of LUM/IVA; the percent predicted forced expiratory volume in 1 s (ppFEV1) and the number of pulmonary exacerbations (PEx) during the three years before up to three years after modulator initiation; and the forskolin-induced swelling (FIS) responses to LUM/IVA, quantified in intestinal organoids.

Results

On a group level, the results showed an acute change in ppFEV1 after LUM/IVA initiation (2.34%, 95%CI 0.85–3.82, p = 0.003), but no significant change in annual ppFEV1 decline in the three years after LUM/IVA compared to the three years before (change: 0.11% per year, 95%CI: −1.94–2.19, p = 0.913). Neither of these two outcomes was associated with any of the candidate predictors on an individual level. The median number of PEx per patient year did not significantly change in the three years after LUM/IVA compared to the years before (median: 0.33/patient year, IQR: 0–0.67 before vs. median: 0/patient year, IQR: 0–0.67 after p = 0. 268). The PEx rate after modulator initiation was associated with the PEx rate before (IRR: 2.26, 95%CI: 1.67–3.08, p < 0.001), with sex (males vs. females IRR: 0.36, 95%CI: 0.21–0.63, p = 0.001) and with SwCl at baseline (IRR: 0.96, 95%CI: 0.94–0.98, p = 0.001). The change in SwCl was also significant (−22.9 mmol/L (95%CI: −27.1–−18.8, p < 0.001) and was associated with SwCl at baseline (−0.64, 95%CI: −0.90–−0.37, p < 0.001) and with sex (males vs. females 8.32, 95%CI: 1.82–14.82, p = 0.013).

Conclusion

ppFEV1 decline after CFTR modulator initiation remains difficult to predict in individual patients in a real-world setting, with limited effectiveness for double CFTR modulator therapies. The PEx rate prior to CFTR modulator treatment initiation, sex and SwCl at baseline could be potential predictors of long-term PEx rate and of changes in SwCl after modulator initiation.

INTRODUCTION

Cystic Fibrosis (CF) is the most prevalent autosomal recessive disorder, that affects over 90,000 people worldwide [1]. It is caused by mutations in the *cystic fibrosis transmembrane conductance regulator* (CFTR) gene, which encodes for an apically expressed anion channel in epithelial cells. The CFTR channel regulates fluid and electrolyte homeostasis of many mucosal surfaces [2]. The most common mutation is the deletion of the amino acid phenylalanine at position 508 (F508del), which is carried by approximately 85% of the global CF population. This mutation results in a misfolded CFTR protein with a strongly reduced apical trafficking and function. People with homozygous F508del mutations can benefit from small molecule combination therapy that targets the distinct defects of the F508del protein. The correctors lumacaftor (LUM, VX-809) and tezacaftor (TEZ, VX-661) enhance the processing and trafficking of the F508del-CFTR protein to the cell surface. Potentiators such as ivacaftor (IVA, VX-770) enhance the channel-opening probability (gating) and further increase the lumacaftor- or tezacaftor-corrected F508del function at the cell surface. Phase 3 clinical trials in people with CF (pwCF) homozygous for the F508del mutation demonstrated a modest efficacy of LUM/IVA or TEZ/IVA, as indicated by a 2.6–4% absolute increase in percent predicted forced expiratory volume in 1 s (ppFEV1) after 24 weeks of treatment, accompanied by a reduction in the pulmonary exacerbation (PEx) rate and sweat chloride concentration (SwCl) [3,4]. Sustained efficacy of treatment was demonstrated in phase 3 open-label extension trials with a two-year follow-up. These trials showed an average absolute reduction in ppFEV1 of 1–1.4% per year [5,6] and a 30–39% lower annualized PEx rate [3–6]. There is, however, a substantial variability in individual clinical response to CFTR modulators and the reason for this remains unknown. This variability has also been observed for newer and more effective triple therapy (elexacaftor/tezacaftor/ivacaftor) in people with F508del [7]. Predicting the CFTR modulator response in pwCF based on individual characteristics and *in vivo* or *in vitro* biomarkers would be useful in order to target costly therapies towards those patients who would benefit.

Biomarkers of CFTR function have been studied for their ability to predict individual clinical response to CFTR modulators. Several studies that focused on forskolin-induced swelling (FIS) of intestinal organoids found strong correlations between the average *in vitro* FIS response to CFTR modulators and short-term clinical drug response across groups with different genotypes [8,9] and in individuals with a variety of CFTR mutations [10]. These results raised interest in using the FIS assay as a biomarker to predict individual clinical responses to CFTR modulating therapies. Within pwCF homozygous for F508del, two small studies failed to predict

the individual short-term clinical response to lumacaftor/ivacaftor (LUM/IVA) based on *in vitro* biomarkers such as FIS, nasal potential difference (NPD) intestinal current measurement (ICM) [11,12], β-adrenegic sweat secretion and serum drug concentration [12]. Other exploratory studies also did not detect an association between individual FIS response and short-term clinical response to LUM/IVA in pwCF carrying an A455E mutation [13] or to IVA in people with residual CFTR-function mutations [14]. Despite significant group-level responses in SwCl [11–14] or ppFEV1 [12,14], correlations between clinical endpoints were absent [12] or not reported [11,13,14].

In this real-world observational cohort study in pwCF homozygous for F508del, we assessed whether long-term ppFEV1 decline, PEx rate and SwCl change in response to LUM/IVA and whether long-term individual outcomes can be predicted by a combination of *in vivo* and *in vitro* biomarkers.

MATERIALS AND METHODS

Study design and population

This multicenter observational cohort study was conducted in the CF centers of the University Medical Center Utrecht (UMCU) and Haga Teaching Hospital in The Hague, both in the Netherlands. PwCF homozygous for the F508del mutation were eligible for this study if CFTR modulating treatment with LUM/IVA had been initiated before July 2018 and individual intestinal organoids had been collected and stored in a biobank prior to CFTR modulator treatment. No exclusion criteria were specified. Total clinical follow-up was six years, starting from three years before up to three years after treatment initiation, or until censoring in case of (1) treatment discontinuation due to adverse events, (2) transition to elexacaftor/tezacaftor/ivacaftor (ETI), (3) lung transplantation, (4) death or (5) participants being lost to follow-up. Transition to TEZ/IVA during the study period was accepted, as its efficacy was considered to be comparable to LUM/IVA [3–6]. Written informed consent was obtained from all participants. The study was approved by the institutional review board (IRB) of the UMCU (IRB #16-668, TcBio #14-008).

Study parameters

Outcomes

The primary outcome was defined as change in average annual lung function decline in the first three years after LUM/IVA initiation, compared to the decline in the three years prior to LUM/IVA. Lung function was expressed as ppFEV1, calculated according to Global Lung function Initiative (GLI) guidelines [15]; ppFEV1 was

routinely measured every 3–6 months.

An acute change in ppFEV1 after LUM/IVA initiation, the total number of pulmonary exacerbations (PEx) requiring intravenous (IV) antibiotics during the first three years after LUM/IVA and a change from baseline SwCl (mmol/L) six months after LUM/IVA initiation were defined as secondary outcomes.

Candidate predictors

FIS response to LUM/IVA, defined as forskolin-induced organoid swelling after incubation with 3 μM forskolin, quantified as area under the curve (AUC) was selected as potential predictor of interest based on prior research. For all outcomes, the following candidate predictors were included based on previous literature and availability: total number of PEx requiring IV antibiotics during the three years before LUM/IVA treatment; sex (male/female); age at baseline, defined as age at date of treatment initiation; and SwCl at baseline (mmol/L), defined as the most recent SwCl value before LUM/IVA initiation. Average ppFEV1 decline during three years prior to LUM/IVA treatment was also included as a potential predictor of the primary outcome, whereas ppFEV1 at baseline was included for the secondary outcomes.

Study procedures

Clinical data collection

Data on clinical study parameters were retrieved from electronic medical records. We collected all available ppFEV1 measurements within the follow-up period. Total number of PEx was counted based on the start and stop dates of IV antibiotic courses in the three years before and three years after LUM/IVA initiation. Additional data were collected regarding type of CFTR modulating treatment and date and reason of censoring, if applicable. Date of treatment initiation was defined by the first start date of LUM/IVA. If LUM/IVA was discontinued within 3 months after the first initiation and/or for at least six months, date of treatment initiation was defined by the re-introduction date of LUM/IVA.

Organoid cultures and measurements

All procedures regarding organoid culturing and measurements were performed by HUB Organoid Technology in the Netherlands. The isolation of crypts out of rectal biopsies, the establishment of intestinal organoids and the FIS assays were performed according to previously described methods [8,16,17]. For the FIS assays, organoids were disrupted and seeded in a 96-well plate with an optical bottom (30–60 organoids per well). Immediately after seeding, 3 μM lumacaftor (VX- 809) was added. After 24 h, organoids were stained with 10 μM calcein green, and 3 μM

ivacaftor (VX-770) and forskolin (0.128 μM) were added. For each organoid model, technical duplicates and biological triplicates were performed (n = 6 datapoints). Organoid size was measured by fluorescence microscopy for a period of 60 min, taking images every 10 min with the Perkin Elmer Operetta CLS microscope. The resulting images were analyzed using Fiji (Fiji Life-Line version, 25 November 2014), an open-source image processing package based on ImageJ. HUB generated a script which recognizes organoids and quantifies change in size over time. The script identifies objects (organoids) and measures the area of each object at each time point. Subsequently, we calculated the change in size over time (relative to t = 0) for each object and the median change of size for each time point. The area under the curve (AUC) of the relative organoid size over time curves was calculated as the cumulative positive area between each two adjacent data points (size at t = 0).

Statistical analysis

Baseline characteristics of the study population were summarized with descriptive statistics. A multivariable linear mixed effects model was used to estimate ppFEV1 decline over time, ranging from −3 years to +3 years, with time = 0 (baseline) set at the date of LUM/IVA initiation. The model included a random intercept per subject and a random slope for time, CFTR modulator treatment, and the interaction between time and CFTR modulator treatment, assuming an unstructured covariance matrix. As fixed effects, we included time (in years) as a continuous variable; CFTR modulator, indicating CFTR modulator treatment status at the time of each ppFEV1 measurement (0 = no CFTR modulator, 1 = LUM/IVA or TEZ/IVA in case of transition during the study period); age at baseline; SwCl at baseline; sex; total number of PEx in the three years before CFTR modulator treatment; and FIS response to LUM/IVA. Moreover, an interaction term for time and CFTR modulator treatment (time: CFTR modulator) was added to the model, representing the change in ppFEV1 decline in the years after LUM/IVA. Subsequently, we used stepwise forward selection to test all other model covariates as a two-way interaction with time to assess whether ppFEV1 decline before CFTR modulator treatment was associated with covariate status, and as a two-way interaction with CFTR modulator to determine whether candidate predictors were associated with the acute change in ppFEV1 after LUM/IVA initiation. Finally, candidate predictors were tested as a three-way interaction with time : CFTR modulator, in order to identify predictors of change in ppFEV1 decline after LUM/IVA initiation. Model performance was assessed based on conditional and marginal R^2.

Subsequently, we used a multivariable negative binomial model to identify predictors of the total number of PEx in the three years after LUM/IVA initiation. This model included total number of PEx in the three years after treatment as an outcome variable, with

sex, age at baseline, SwCl at baseline, ppFEV1 at baseline, (log-transformed) number of PEx in the three years before LUM/IVA and FIS response to LUM/IVA as potential predictors. Total follow-up time (in years) after LUM/IVA initiation was used as offset. Model performance was assessed based on Nagelkerke's R^2.

Finally, the change in SwCl after LUM/IVA was analyzed with a multivariable linear regression model, including SwCl at baseline, age at baseline, ppFEV1 at baseline, sex, PEx in the three years before treatment initiation and FIS response to LUM/IVA as predictor variables. Adjusted R^2 was used to describe model performance.All analyses were performed in complete cases, given the low proportion of missing data (3% missing SwCl at baseline, 7% missing SwCl after LUM/IVA). *p*-values < 0.05 were considered statistically significant. Statistical packages lme4, lmerTest, MASS and Performance of R version 4.1.1 for Mac were used for the analyses.

RESULTS

Study population

In total, 97 pwCF with the F508del/F508del mutation were included in this study. Mean follow-up time was 3.2 years (±0.6 SD) before and 2.7 years (±0.7 SD) after LUM/IVA initiation. Censoring occurred in 12 participants due to transition to ETI (*n* = 9), treatment discontinuation (*n* = 1) or being lost to follow-up (*n* = 2). As summarized in **table 1**, a substantial proportion (68%) of the study population transitioned to TEZ/IVA during the follow-up period, which was on average after two years of treatment with LUM/IVA (mean 1.9 years ± 0.5 SD). Over the entire study period, 2332 ppFEV1 measurements were collected from all participants. SwCl at baseline and SwCl after LUM/IVA were missing in 3 (3%) and 7 (7%) participants, respectively.

ppFEV1 and change in ppFEV1 decline

A multivariable linear mixed effects model was used to assess ppFEV1 decline over the entire observation period and to identify predictors of the acute change in ppFEV1 and of ppFEV1 decline in the three years after LUM/IVA initiation. Three participants were excluded from the analysis due to missing SwCl at baseline. As shown in **table 2**, average annual ppFEV1 decline before LUM/IVA initiation was –2.14% per year (95% CI 3.77––0.51, *p* = 0.012). A significant acute improvement of ppFEV1 was observed after LUM/IVA initiation (2.34%, 95% CI 0.85–3.82, *p* = 0.003), but the average annual ppFEV1 decline over three years did not change compared to the years before (change in decline: 0.11% per year, 95% CI –1.94–2.19, *p* = 0.913).

Table 1. Baseline characteristics of the study population (n=97)

CF center, n (%)	
University Medical Center Utrecht	88 (91)
Haga Teaching Hospital The Hague	9 (9)
CFTR modulator, n (%)	
lumacaftor/ivacaftor	97 (100)
tezacaftor/ivacaftor transition during follow-up	66 (68)
CFTR modulator treatment duration (years), mean (SD)	2.7 (0.7)
ppFEV1 3 years before modulator (%), mean (SD)	69.6 (21.8)
ppFEV1 at baseline (%), mean (SD)	66.4 (22.0)
Number of PEx per patient year before modulator, median (IQR)	0.33 (0–0.67)
SwCl at baseline (mmol/L), mean (SD)	92.0 (13.1)
Female sex, n (%)	44 (45)
Age at baseline (years), median (IQR)	23.5 (17.0–31.1)
FIS response to LUM/IVA (AUC), median (IQR)	1.9 (647.9 - 1418.1)

CFTR: Cystic Fibrosis Transmembrane conductance Regulator protein. Baseline: defined as date of CFTR modulator initiation. ppFEV1: percent predicted forced expiratory volume in 1 second. Number of PEx: average number of pulmonary exacerbations (PEx) requiring intravenous (IV) antibiotics per patient year, in the three years before CFTR modulator initiation. SwCl: sweat chloride concentration. FIS response to LUM/IVA: corrected forskolin-induced swelling response of intestinal organoids to 3 μM lumacaftor/ivacaftor (LUM/IVA) and 0.128 μM forskolin minus the response to 0.128 μM forskolin alone, quantified as area under the curve (AUC) of the normalized organoid swelling over 1 h.

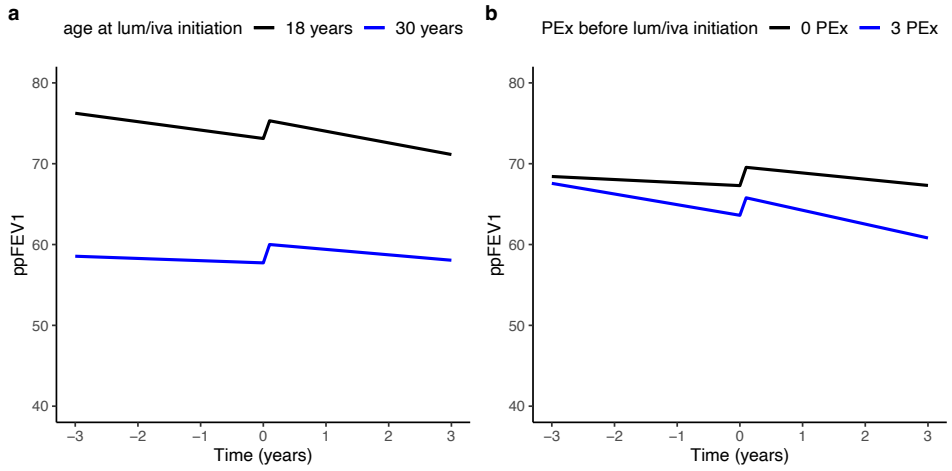
Table 2. Multivariable linear mixed effects model of ppFEV1 decline (n=94, obs=2233)

	Coefficient	95% CI	P-value
Time	-2.14	-3.77 – -0.51	0.012*
CFTR modulator	2.34	0.85 – 3.82	0.003*
Male sex	6.38	-0.29 – 13.06	0.069
Age at baseline	-1.28	-1.66 – -0.91	<0.001*
SwCl at baseline	0.32	0.06 – 0.58	0.017*
Number of PEx	-1.22	-2.83 – 0.38	0.145
FIS response to LUM/IVA	0.19	-0.42 – 0.79	0.554
Time : age at baseline	0.06	0.02 – 0.11	0.004*
Time : number of PEx	-0.31	-0.48 – -0.14	<0.001*
Time : CFTR modulator	0.11	-1.94 – 2.19	0.913

Definitions: Time in years. CFTR modulator indicates treatment with LUM/IVA or TEZ/IVA (in case of transition during the study follow-up). Male sex compared to the reference category female sex. Age in years. SwCl in mmol/L. Number of PEx: total number of PEx requiring IV antibiotics in the three years before CFTR modulator initiation. FIS response to LUM/IVA: corrected forskolin-induced swelling response of intestinal organoids to 3 μM lumacaftor/ivacaftor (LUM/IVA) and 0.128 μM forskolin minus the response to 0.128 μM forskolin alone, quantified as area under the curve (AUC) of the normalized organoid swelling over 1 h, scaled 1:100. Model performance: conditional R^2 0.95, marginal R^2 0.34. Interpretation: The coefficient of time reflects the average annual ppFEV1 decline over time before treatment initiation. The coefficient CFTR modulator represents the acute change in average ppFEV1 directly after modulator initiation. The coefficients of male sex, age at baseline, SwCl at baseline, number of PEx and FIS response to LUM/IVA illustrate the associations with average ppFEV1. Coefficients of time:age at baseline and time:number of PEx define the association of age and PEx with ppFEV1 decline before CFTR modulator initiation. Time:CFTR modulator indicates the average change in ppFEV1 decline after CFTR modulator initiation compared to the ppFEV1 decline before modulator use.

We determined whether candidate predictors (sex, age, SwCl, PEx and FIS response to LUM/IVA) were associated with average ppFEV1 and ppFEV1 decline before treatment with LUM/IVA. Age and SwCl at baseline demonstrated a significant association with average ppFEV1 (−1.28, 95% CI −1.66–−0.91, $p < 0.001$ and 0.32, 95% CI 0.06–0.58, $p = 0.017$, respectively). Age at baseline and total number of PEx were associated with ppFEV1 decline before LUM/IVA initiation and were therefore included in the multivariable linear mixed effects model (**table 2**). **Figure 1a** illustrates that predicted ppFEV1 decline before LUM/IVA was on average 0.06% per year (95% CI 0.02–0.11, $p = 0.004$, **table 2**) less for every additional year in age at baseline. In addition, predicted annual ppFEV1 decline before LUM/IVA was on average 0.31% per year (95% CI −0.48–−0.14, $p < 0.001$, **table 2**) stronger per experienced PEx (**figure 1b**).

Figure 1. Predicted ppFEV1 decline before and after CFTR modulator initiation at varying ages and number of PEx



Plots are based on the linear mixed effects model coefficients in **table 2** to illustrate the associations of age at baseline and PEx with ppFEV1 decline. Time ranges from -3 years before to +3 years after LUM/IVA initiation, with time=0 (baseline) defined by the start date of treatment with LUM/IVA. Model estimates suggested a faster ppFEV1 decline at a younger age which diminished at an older age. This is illustrated for an age at baseline of 18 years and 30 years (a), while all other covariates were kept constant at their mean or median values or at the reference category (as reported in **table 1**). In addition, predicted ppFEV1 decline was plotted for pwCF without PEx vs. with 3 PEx in the three years before LUM/IVA initiation (b), to illustrate that predicted ppFEV1 decline may deteriorate with an increasing number of PEx. Model performance: conditional R^2 0.95, marginal R^2 0.34.

In contrast to the moderate significant acute improvement after LUM/IVA initiation, the three-year average annual ppFEV1 decline did not change on a group level when comparing trends before and after LUM/IVA treatment. Nevertheless, we did not find

an association of either the acute change in ppFEV1 or annual ppFEV1 decline in the three years after LUM/IVA initiation with the candidate predictors, which were left out of the model in order to reduce the complexity of the model and to improve the performance (conditional R^2 0.95, marginal R^2 0.34).

Pulmonary exacerbations

Overall, the median number of PEx requiring IV antibiotics in the three years before LUM/IVA initiation was 0.33 (IQR: 0–0.67) per patient year, which did not significantly change in the three years after (median: 0, IQR: 0–0.67, p = 0.268). Predictors of the absolute number of PEx during the first three years of treatment with LUM/IVA were assessed with a negative binomial model. Three participants were excluded due to missing SwCl at baseline.

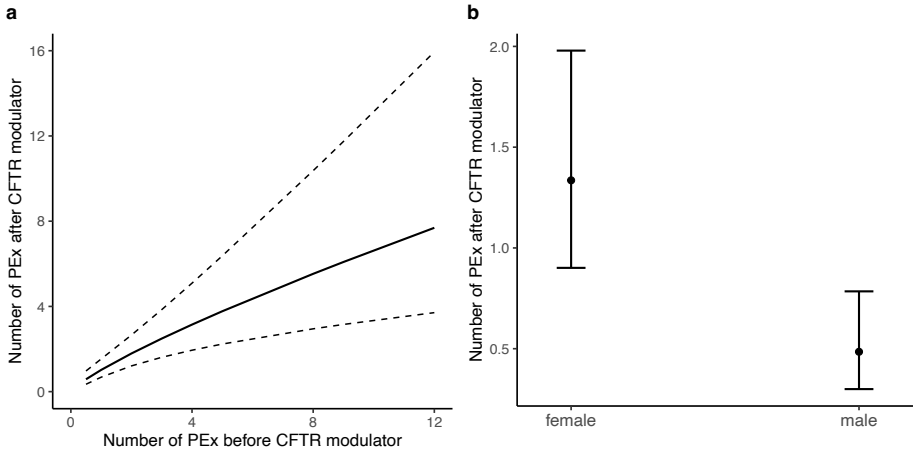
The number of PEx after LUM/IVA was associated with the (log-transformed) number of PEx before LUM/IVA, with sex and SwCl at baseline (**table 3**). The predicted relationship between the number of PEx before and after LUM/IVA on the original scale is illustrated in **figure 2a**. Relative rate of PEx in males was three times lower (IRR 0.36, 95% CI 0.21–0.63, p < 0.001) compared to females (**figure 2b**). SwCl at baseline was also significantly associated with the number of PEx after LUM/IVA (IRR 0.96, 95% CI 0.94–0.98, p = 0.001), but not with age at baseline, ppFEV1 at baseline or FIS response to LUM/IVA.

Table 3. Multivariable negative binomial model of total number of PEx requiring IV antibiotics in the first three years after LUM/IVA initiation (n=94)

	Coefficient	IRR	95% CI (IRR)	P-value
Log(number of PEx)	0.81	2.26	1.67 – 3.08	<0.001*
Male sex	-1.01	0.36	0.21 – 0.63	<0.001*
Age at baseline	0.03	1.03	0.99 – 1.06	0.125
SwCl at baseline	-0.04	0.96	0.94 – 0.98	0.001*
ppFEV1 at baseline	-0.01	0.99	0.98 – 1.01	0.467
FIS response to LUM/IVA	0.01	1.01	0.69 – 1.06	0.706

Interpretation: Coefficients are on the log-scale. The incidence rate ratios (IRR) are the coefficients transformed back to the original scale and represent the relative change in the number of PEx in the three years after CFTR modulator initiation for every 1-unit change of the continuous variables or for male sex compared to the reference category female sex. Model performance: Nagelkerke's R^2=0.60.

Figure 2. Association of PEx in the three years after LUM/IVA initiation with the number of PEx three years before LUM/IVA (2a) and the difference between females and males (2b).



Predicted associations are illustrated on the original scale based on the incidence rate ratios (IRR) in **table 3**. All other covariates were kept constant at their mean or median values or at the reference category (as reported in **table 1**). Dashed lines in panel a) and error bars in panel b) represent 95% confidence intervals. Model performance: Nagelkerke's R^2=0.60.

Change in sweat chloride concentration

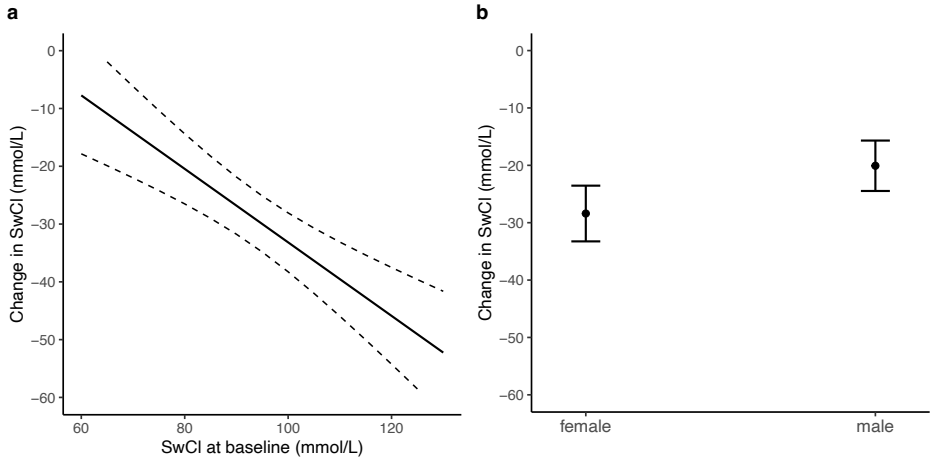
SwCl levels significantly improved after approximately six months (mean 7.2 months ± 4.8 SD) of treatment with LUM/IVA, with an average absolute change from baseline of −22.9 mmol/L (95% CI −27.1−−18.8, $p < 0.001$). Candidate predictors of change in SwCl were assessed by means of linear regression. We excluded 9 participants due to missing SwCl at baseline or SwCl after treatment initiation. **Table 4** shows that the change in SwCl after LUM/IVA was associated with SwCl at baseline (−0.64, 95% CI −0.90−−0.37, $p < 0.001$) and sex (8.32, 95% CI 1.82–14.82, $p = 0.013$). As illustrated in **figure 3**, this suggested that the decrease in SwCl was greater in participants with higher baseline SwCl levels (**figure 3a**) and smaller in males compared to females (**figure 3b**). The change in SwCl after treatment initiation was not associated with baseline ppFEV1, PEx or FIS response to LUM/IVA.

Table 4. Multivariable linear regression model of absolute change in SwCl after LUM/IVA (n=88)

	Coefficient	95% CI	P-value
SwCl at baseline	-0.64	-0.90 – -0.37	<0.001*
Age at baseline	0.05	-0.37 – 0.47	0.822
ppFEV1 at baseline	-0.02	-0.20 – 0.15	0.786
Male sex	8.32	1.82 – 14.82	0.013*
Number of PEx	-0.40	-1.95 – 1.15	0.612
FIS response to LUM/IVA	0.22	-0.31 – 0.74	0.411

Definitions: FIS response to LUM/IVA: corrected forskolin-induced swelling response of intestinal organoids to 3 μM lumacaftor/ivacaftor (LUM/IVA) and 0.128 μM forskolin minus the response to 0.128 μM forskolin alone, quantified as area under the curve (AUC) of the normalized organoid swelling over 1 h, scaled 1:100.
Interpretation: Model coefficients represent the predicted change in SwCl for every 1-unit change of the continuous variables or for male sex compared to female sex (which is the reference category). Model performance: adjusted R^2: 0.26.

Figure 3. Association of change in SwCl six months after LUM/IVA with SwCl at baseline (3a) and the difference between females and males (3b)



Predicted associations are illustrated according to model coefficients in **table 4**. All other covariates were kept constant at their mean or median values or at the reference category (as reported in **table 1**). Dashed lines in panel a) and error bars in panel b) represent 95% confidence intervals. Model performance: adjusted R^2=0.26.

DISCUSSION

The aim of the study presented here was to assess the real-world long-term clinical effectiveness of double CFTR modulator therapies in pwCF homozygous for the F508del mutation and to assess the association of several *in vivo* and *in vitro* parameters with clinical endpoints, in order to determine whether these parameters could serve as predictors of long-term treatment response to CFTR modulators in a real-world setting.

This study did not show a significant improvement in ppFEV1 decline or the number of PEx, when comparing three years before and after treatment with LUM/IVA. However, the data did indicate an overall acute ppFEV1 improvement and a decline in SwCl concentration after six months, consistent with previous observations in clinical trials [3–6]. Our results of ppFEV1 decline were different from the original phase 3 open-label extension study of LUM/IVA in pwCF homozygous for the F508del mutation, which showed an average annual ppFEV1 decline of −1.3% compared to −2.3% in untreated matched historical controls after 120 weeks of treatment [5]. This is probably related to our real-world approach, which had no restrictions regarding age or baseline ppFEV1 and a longer follow-up period before and after treatment initiation. One other study reported a moderate improvement of ppFEV1 decline after one year of LUM/IVA in a real-world setting [18], which is in line with the short-term average improvement of 2.3% found in our study population and in the first short-term clinical trials [3,4]. These findings underline the risk of extrapolating data from controlled studies into daily clinical practice. Alternatively, the real-world setting may be responsible for stronger variations in ppFEV1 measurements. We aimed to reduce the impact of measurement variability of ppFEV1 by including multiple repeated measurements, but other unmeasured sources of variation in ppFEV1 may play an important role [19].

Despite the absence of an overall group-level change in ppFEV1 decline, associations at the individual level could still be demonstrated when substantial individual variation is present. This study showed that ppFEV1 decline before CFTR modulator use was associated with age and number of PEx, but we could demonstrate neither an association between any of the studied parameters and the acute change in ppFEV1, nor an association between the parameters and the change in long-term ppFEV1 decline after CFTR modulator initiation. This suggests that the individual variation in both the acute change in ppFEV1 and the change in ppFEV1 decline might have been too low in combination with the limited effectiveness of LUM/IVA.

The number of PEx in the three years before CFTR modulator treatment and sex were associated with the number of PEx in the three years after CFTR modulator initiation. This is in accordance with several studies reporting worse pulmonary outcomes and a higher mortality risk in females [20–22], despite equal levels of care between males and females [23]. The so-called 'gender-gap' in pwCF has already been described for many years and the cause is probably multifactorial. The level of female sex hormones may play an important role in the severity of CF lung disease, as it influences mucociliary clearance, infection and inflammation, which ultimately leads to a higher frequency of PEx and a more rapid deterioration of lung function [24]. Since our results did not show a significant reduction in the number

of PEx after LUM/IVA initiation compared to the years before treatment, this could also reflect prognostic differences related to disease severity, which may indicate that those with severe disease manifestations might also remain the most affected patients after treatment initiation.

We found a different association between sex and change in SwCl after six months of LUM/IVA, with a greater reduction in SwCl in females compared to males. So far, few studies have focused on the differential effect of CFTR modulators between sexes on clinical outcomes. One study in a small group of pwCF with severe lung disease (ppFEV1 < 40%) described no differences in PEx rate between males and females one year after commencement with LUM/IVA [25]. However, a greater reduction in both SwCl and PEx rate was observed in females carrying CFTR-gating mutations after two years of treatment with IVA [26]. These contradictory results emphasize that additional research is warranted to further elucidate the effects of suggested sex differences on long-term outcomes. Finally, the negative association between SwCl at baseline and the number of PEx in the years after LUM/IVA initiation, although it was very weak, was opposed to existing literature, which has reported that higher levels of SwCl correspond to a more severe CF phenotype [27,28]. The decreasing number of exacerbations after LUM/IVA with increasing baseline SwCl levels are, therefore, difficult to explain and might have been influenced by measurement variation in SwCl [29].

To date, only a few studies have focused on the association of *in vitro* biomarkers such as FIS, ICM and NPD with short-term changes in ppFEV1 and SwCl as parameters of clinical response to CFTR modulators. Even though residual CFTR function measured by FIS was correlated with disease severity of pwCF homozygous for F508del [28], our results were in line with other exploratory studies, which were also not able to detect associations between individual short-term clinical response and *in vitro* biomarkers quantifying response to CFTR modulators [11–14]. This is likely explained by the limited effectiveness of LUM/IVA and the relatively low individual variation in the measured clinical outcomes in a real-world setting in a homogeneous group of F508del/F508del pwCF. Other studies, which demonstrated a strong association of FIS with short-term changes in SwCl and ppFEV1 on a group level and on the individual level, included pwCF with a variety of CFTR mutations and a wider range of clinical responses [8–10]. Future research might therefore focus on the prediction of clinical response to highly effective CFTR modulators such as ETI or other more potent therapies, and may include people with a variety of CFTR mutations to identify clinical responders and facilitate personalized treatment.

The retrospective observational design of this real-world before-after study could be regarded as a limitation of this study, although all study parameters were systematically collected as part of standard clinical care with a low proportion of missing data. In addition, a substantial proportion (63%) of our participants transitioned to TEZ/IVA after approximately two years of treatment with LUM/IVA. This could have influenced the results, but we expect that this did not over- or underestimate the change in ppFEV1 decline given the comparable efficacy of these CFTR modulators [4,6]. Unfortunately, the number of selected candidate predictors was restricted by the sample size. Larger prospective studies including more candidate predictors would be required to be able to develop and validate a clinical prediction model.

In summary, our study showed a limited overall effectiveness of double CFTR modulator therapy in pwCF homozygous for F508del after three years. Individual prediction of long-term clinical response remains difficult in a real-world setting, although PEx rate prior to CFTR modulator treatment initiation, sex and SwCl at baseline could be potential predictors of long-term PEx rate and of changes in SwCl after modulator initiation.

Acknowledgements
We would like to thank all people with CF who participated in this study; the clinical research teams of the UMC Utrecht and Haga Teaching Hospital The Hague for the recruitment of participants and the collection of clinical data; and the research team of HUB Organoid Technology for the culturing and measurements of intestinal organoids.

Funding
This research received no external funding.

Author contributions
Conceptualization: D.M., P.v.M., H.H., J.B. and K.v.d.E.; methodology: D.M., R.v.d.B., R.E., J.B. and K.v.d.E.; formal analysis: D.M., J.K., R.v.d.B. and R.E.; data curation: D.M., J.K. and R.E.; writing—original draft preparation: D.M., M.B. and K.v.d.E.; writing—review and editing: D.M., M.B., P.v.M., J.K., R.v.d.B., R.v.d.M., H.H., R.E., J.B. and K.v.d.E.; visualization: D.M. and R.E.; supervision: R.E., J.B. and K.v.d.E. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest
K.v.d.E. reports grants from GSK, as well as grants from Nutricia, TEVA, Gilead, Vertex, ProQR, Proteostasis, Galapagos NV and Eloxx, outside the submitted

work; in addition, K.v.d.E. has a patent 10,006,904 with royalties paid. J.B. reports personal fees from Vertex Pharmaceuticals, Proteostasis Therapeutics, Eloxx Pharmaceuticals, Teva Pharmaceutical Industries and Galapagos, outside the submitted work; in addition, J.B. has a patent related to the FIS-assay with royalties paid. All other authors declare no conflict of interest.

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CHAPTER 6

Development and validation of a novel personalized electronic patient-reported outcome measure to assess quality of life (Q-LIFE): a prospective observational study in people with Cystic Fibrosis

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eClinicalMedicine 2023, Jul 27;62:102116

SUMMARY

Background

Generic and disease-specific patient-reported outcome measures (PROMs) may lack relevance and sensitivity on a patient-level in chronic diseases with differential disease expression and high individual variability, such as Cystic Fibrosis (CF). This study aimed to develop and validate a novel personalized electronic PROM (ePROM) that captures relevant aspects of quality of life in individuals with CF.

Methods

The Q-Life app was developed as a short personalized ePROM to assess individual quality of life. Psychometric properties were assessed in a single-center cross-sectional study between September 2019 and September 2021 and in a prospective cohort study between September 2021 and September 2022.

Findings

Combined studies included 223 participants (median age: 24 years, IQR: 19·0–32·5 years, range: 12·0–58·0 years). Internal consistency (Cronbach's alpha: 0·83–0·90) and test-retest reliability (intraclass correlation coefficient: 0·90; 95%CI: 0·65–0·92; $p<0·001$) of quality of life (Q-Life) scores were strong. Q-Life scores were associated with overall Cystic Fibrosis Questionnaire-Revised (CFQ-R) scores ($\rho=0·71$; $p<0·001$), CFQ-R respiratory domain scores ($\rho=0·57$; $p<0·001$) and forced expiratory volume in 1s ($\rho=0·41$; $p<0·001$). Furthermore, Q-Life scores improved from 65·0 (IQR: 45·0–63·3) at baseline to 84·2 (IQR: 75·0–95·0) and 87·5 (IQR: 75·0–100·0) after 3 and 6 months of elexacaftor/tezacaftor/ivacaftor treatment (change: 20·8; 95%CI: 17·5–25·0; $p<0·001$), comparable to CFQ-R respiratory domain scores (change: 22·2, 95%CI: 19·4–25·0, $p<0·001$).

Interpretation

The Q-Life app is a reliable, valid and sensitive personalized ePROM to measure all aspects of quality of life that really matter to individuals with Cystic Fibrosis. This patient-centered approach could provide important advantages over generic and disease-specific PROMs in the era of personalized medicine and value-based healthcare.

Funding

Dutch Cystic Fibrosis Foundation, Health Holland.

RESEARCH IN CONTEXT

Evidence before this study

Patient-reported outcome measures (PROMs) play an important role in clinical trials and contribute to the transition towards a more value-based and patient-centered healthcare system. Traditional generic and disease-specific PROMs may lack relevance and sensitivity on a patient-level in chronic diseases such as Cystic Fibrosis (CF), due to heterogeneous disease manifestations and disparities in treatment options for people with different disease characteristics, leading to highly variable individual life perspectives. Personalized PROMs may better capture the broader impact of disease, new treatment modalities and healthcare on individual patients, yet such personalized tools have not been developed and validated so far. We searched PubMed using the query "(patient-reported outcome measure[MeSH Terms]) OR (patient-reported outcome[MeSH Terms])) AND (cystic fibrosis[MeSH Terms]" for articles published up to March 6th, 2023. No language restrictions were used. Reference lists and related articles were also screened for additional relevant studies. The search identified 26 articles reporting of generic and disease-specific PROMs in CF, including two recent reviews summarizing all PROMs used in CF research and care. These reviews showed that the Cystic Fibrosis Questionnaire-Revised (CFQ-R) is by far the most commonly used and best validated disease-specific PROM, but also emphasized the urgent need for a novel, more relevant and patient-centered electronic PROM that allows for remote monitoring.

Added value of this study

This is the first study, to our knowledge, describing a personalized electronic PROM (Q-Life app) that is able to capture all aspects of quality of life that matter to individual patients. The app was validated in a cohort of 223 people with CF of a wide age range with different genotypes, varying disease manifestations and treatments. This personalized PROM may provide important advantages over traditional generic and disease-specific PROMs as it is short, electronic and solely focused on items that are meaningful and relevant to individual patients.

Implications of all the available evidence

This first validation study demonstrated the value of a personalized PROM to assess the impact of CF disease and highly effective CFTR modulator treatment on quality of life of individuals with CF. Future studies should be performed to assess external validation of the Q-Life app in different CF populations and settings and to elucidate the potential of a personalized PROM for other chronic diseases.

INTRODUCTION

The importance of adequate patient-reported outcome measures (PROMs) that are able to capture relevant health benefits from a patient's perspective is increasingly acknowledged in medical research and healthcare. Appropriate validation, reporting and application of PROMs can support pharmaceutical labeling claims, facilitate treatment reimbursement, assist in shared-decision making and contribute to the transition towards a more value-based and patient-centered healthcare system [1–7]. Furthermore, there is a growing need for sufficiently validated remote-monitoring tools such as electronic PROMs (ePROMs), as the digitalization in medical research and care has rapidly gained momentum since the COVID-19 pandemic.

PROMs can be defined as questionnaires that collect information on health status, as experienced and reported directly by the patient [2]. Over the last decades, numerous generic and disease-specific PROMs have been developed, which are generally focused on symptoms, treatment satisfaction, functional status or health-related quality of life [8]. Although disease-specific PROMs are considered to be more sensitive and reflective of patient symptoms and functioning than generic PROMs [3], disease-specific PROMs are still composed of a fixed list of questions related to pre-defined domains that may lack relevance and sensitivity on a patient-level. Moreover, the growing number of disease-specific PROMs hampers comparability of outcomes among patients with different diseases.

Sporadically, patient-specific outcome measures such as goal-attainment scaling have been developed and applied in different medical disciplines [9–13]. In goal-attainment scaling (GAS), individual treatment goals are defined together with the patient's healthcare team, whereas scoring is performed by an independent assessor [11]. Consequently, this method does not fulfill the criteria of a PROM, yet it has been demonstrated that individualized approaches such as GAS can be meaningful and sensitive to systematically measure the impact of treatment modalities and healthcare in a patient-centered way [9,10,14].

Personalized approaches can be particularly useful for chronic diseases with heterogeneous clinical manifestations, such as Cystic Fibrosis (CF). CF is a rare genetic multi-system disease that causes severe symptoms and progressive functional loss of e.g. the respiratory and digestive tract. This can have a profound but varying impact on quality of life, depending on the severity, type and progression of disease manifestations as well as on available treatment options [15]. CF could be considered as a model of other chronic diseases for which an effective new

treatment was introduced recently [16,17].

In this study, we aimed to develop a short personalized ePROM that is able to capture all important aspects of quality of life on an individual level, which we validated in people with CF (pwCF).

METHODS

Q-Life app development and features

The Q-Life app was developed in close collaboration with pwCF and parents of children with CF, who were invited and recruited by the Dutch Cystic Fibrosis Foundation (NCFS). The development process is summarized in the **supplementary methods**.

Supplementary figure 1 illustrates how the Q-Life app was used in this study. In the app, users can describe three to five items they find important for their personal quality of life in an open text field, and rank these items in order of importance (**supplementary figure 1a**). Each item has to be labeled with the most appropriate category, which can be selected from a pre-defined list. Subsequently, users can score for each item to what degree they currently feel limited by CF on a 5-point Likert scale, ranging from 1 (almost completely limited) to 5 (not limited), as shown in **supplementary figure 1b**. If desired, the app supports real-time visualization of results **supplementary figure 1c**). Other features include a profile page to collect demographic variables and a brief stepwise manual. Time to complete demographics and compose the personal set of quality of life items takes approximately 5-10 minutes, whereas scoring only takes about 1 minute. The app was downloaded from the Apple store and Google Play store. Online instruction videos are available in Dutch (https://youtu.be/986zX9Z_Cqo) and English (https://youtu.be/3dNTdeI2TYE) and can be found on YouTube by searching "Q-Life CF". The Q-Life app and accompanying software is compliant with international data protection guidelines (ISO27001, NEN7510, ISAE3000).

Study design, population and procedures

This study consisted of two phases. First, we conducted a cross-sectional study in clinically stable people with a confirmed diagnosis of CF aged 14 years and older. Participants were recruited between September 2019 and September 2021, during a routine visit to the outpatient adult or pediatric CF clinic of the University Medical Center (UMC) Utrecht in the Netherlands. In this cross-sectional study, participants composed a personal set of three to five self-described quality

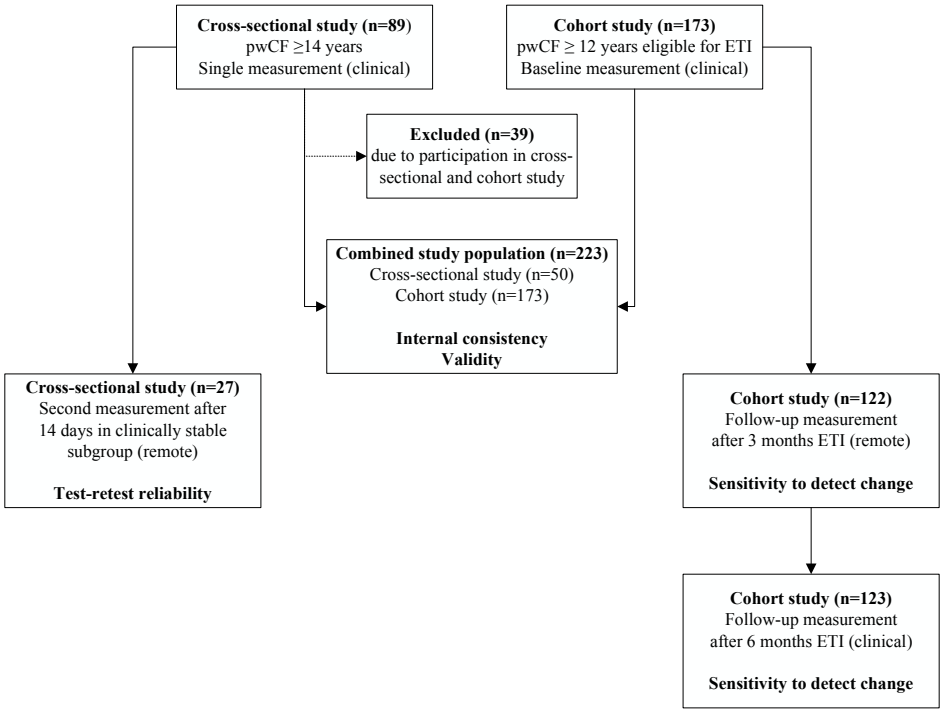
of life items labeled with the most appropriate category and performed a single measurement to what degree they currently felt limited by CF. A clinically stable subgroup was asked to complete a second measurement after 14 days (**figure 1**). In the second phase, pwCF aged 12 years and older who were eligible for treatment with elexacaftor/tezacaftor/ivacaftor (ETI) were enrolled in a prospective observational cohort study between September 2021 and September 2022 in the UMC Utrecht. These participants were asked to describe, label and score their personal quality of life items (Q-Life items) at the baseline clinical visit prior to ETI initiation, remotely after 3 months and during a clinical visit after 6 months of treatment (**figure 1**).

Evaluation of scale reliability and validity was based on the cross-sectional study and the baseline data of the prospective cohort study, whereas sensitivity to detect change was solely derived from the cohort study (**figure 1**).

Additional demographic and clinical data were collected at each study visit, including age, educational level, *Cystic Fibrosis Transmembrane Conductance Regulator protein* (CFTR) genotype, prior CFTR modulator use, lung function, expressed as Forced Expiratory Volume in 1s percentage predicted (FEV1%pred), calculated according to Global Lung function Initiative guidelines [18] and the Cystic Fibrosis Questionnaire-Revised, which is currently the reference standard of CF disease-specific quality of life. [19] In addition, we collected the total number of pulmonary exacerbations (PEx) requiring intravenous (IV) antibiotic treatment in the year prior to the baseline visit, defined as IV-treated PEx.

Psychometric properties of the Q-Life app were assessed in a cross-sectional study including pwCF aged 14 years and older and in a prospective cohort study in pwCF aged 12 years and older who were eligible for ETI. As 39 individuals with CF participated in both studies, their data collected in the cross-sectional study were excluded from the analyses in the combined study population.

Figure 1. Flowdiagram of study design and population



Statistical analysis

Descriptive statistics were used to summarize participant characteristics and personal Q-Life items. Per participant, overall Q-Life scores were calculated for every completed measurement in the Q-Life app. This overall Q-Life score was standardized on a 0- to 100-point scale, calculated by the sum of scores for each self-described quality of life item, expressed as percentage of the maximum possible score. To evaluate the psychometric properties of the Q-Life app, we assessed reliability and validity of Q-Life scores in the combined study population, including data of the cross-sectional study and baseline data of the cohort study participants. If pwCF participated in both studies, we only included their cohort study baseline data in the analyses (**figure 1**). Sensitivity to detect change was evaluated in the cohort study (**figure 1**).

Reliability was assessed by means of internal consistency using Cronbach's α. In addition, test-retest reliability of Q-Life scores was evaluated in the clinically stable participants of the cross-sectional study who completed a second measurement in the Q-Life app after 14 days. In this subgroup (n=27), intraclass correlation coefficients (ICC) were calculated for overall Q-Life scores (average measures), and

for the separate scores of the first three personal Q-Life items (single measures) using a two-way mixed model for absolute agreement [20]. As only a limited number of this subgroup defined a fourth (n=14) and fifth (n=7) personal Q-Life item, sample size was too low to calculate ICCs for these last two separate items.

Content validity could only be assessed on an individual level, because the content of Q-Life items varies across participants and setting. As participants described individual Q-Life items that were important and relevant to their personal situation, the content was verified by a member of the study team during the first study visit. To assess construct validity, we calculated Spearman's correlation coefficients (Spearman's rho = ρ) of overall Q-Life scores with FEV1%pred, CFQ-R respiratory domain scores and with overall CFQ-R scores, which was calculated by the mean of the twelve CFQ-R domain scores. This overall CFQ-R score is not a standard procedure of the CFQ-R scoring, but was added in this study to provide a complimentary score that extends beyond one specific subdomain, with the aim to improve comparability with overall Q-Life scores which are derived from varying categories (i.e. domains) per participant. Subgroup analyses were conducted to estimate the impact of age and sex on the strength of these associations. In addition, we assessed the difference in median overall Q-Life scores between participants who experienced at least one IV-treated PEx and those without IV-treated PEx, between children aged 12-18 years and adults ≥18 years and between females and males (unpaired Wilcoxon's signed rank test). Similar analyses were performed for CFQ-R respiratory domain scores and overall CFQ-R scores.

Finally, we assessed sensitivity to detect change by calculating the absolute change in overall Q-Life scores before and 3 and 6 months after commencement with ETI in complete cases (Paired Wilcoxon's signed rank test), in relation to the change in CFQ-R respiratory domain and overall CFQ-R scores. Absolute changes per individual Q-Life item were summarized by category. A p-value <0·05 was considered statistically significant. All hypothesis tests were two-sided. All analyses were performed in R version 4.3.0.

Ethics statement

All participants provided written informed consent for this study, which was approved by the Institutional Review Board of the UMC Utrecht (#16-668 and #19-344).

Role of the funding source

The Dutch Cystic Fibrosis Foundation (NCFS) recruited patient representatives who were actively involved in the development process of the Q-Life app. Furthermore,

Domenique D. Zomer reviewed the manuscript on behalf of the NCFS. The NCFS and Health Holland had no role in the study design or in the collection, analysis and interpretation of the data. The NCFS and Health Holland also did not have access to the dataset and had no role in the decision to submit the manuscript for publication.

RESULTS

Study population

In total, 89 participants enrolled in the cross-sectional study. Of this group, 27 clinically stable participants performed a second measurement in the Q-Life app after 14 days (median: 14 days, IQR: 14·0–14·5 days). The cohort study included 173 participants. As 39 individuals with CF participated in both studies, we excluded their measurements from the cross-sectional study. This resulted in a total of 223 study participants in the overall analysis (**figure 1**). The study population represented people with a wide range of age (median: 24 years, IQR: 19·0–32·5 years, range: 12·0–58·0 years), a variety of *CFTR* genotypes and prior use of different CFTR modulators (**table 1**). Median overall Q-Life score at study enrollment was 66·7 (IQR: 50·0–87·5). At baseline, ceiling effects were observed in 12% of the participants who obtained the maximum overall Q-Life score of 100, compared to 4% with the maximum CFQ-R respiratory domain score of 100.

Table 1. Baseline characteristics

	Combined study population (n=223)
Cross-sectional study, no.	89
Cohort study, no.	173
Both studies, no.	39
Total included in final analysis, no.	223
CFTR genotype, no (%)	
Homozygous F508del	154 (69·1)
F508del/MF	41 (18·4)
F508del/RF	8 (3·6)
F508del/gating	6 (2·7)
F508del/unknown	6 (2·7)
MF/MF	6 (2·7)
MF/RF	1 (0·4)
MF/unknown	1 (0·4)
CFTR modulator treatment[a], no. (%)	
None	51 (22·9)
Ivacaftor	6 (2·7)
Lumacaftor/ivacaftor	57 (25·6)
Tezacaftor/ivacaftor	92 (41·3)

Table 1. Continued

	Combined study population (n=223)
Elexacaftor/tezacaftor/ivacaftor	17 (7·5)
Sex, no. (%)	
Female	108 (48·4)
Male	115 (51·6)
Level of education, no. (%)	
None	3 (1·3)
Lower/elementary school	8 (3·6)
Preparatory secondary vocational school	27 (12·1)
Secondary vocational school	63 (28·3)
Secondary school	31 (13·9)
Higher professional education	54 (24·2)
University	33 (14·8)
Missing	4 (1·8)
Age (years), median (IQR; range)	24·0 (19·0–32·5; 12·0–58·0)
Age category, no. (%)	
12-18 years	45 (20·2)
≥18 years	178 (79·8)
FEV1%pred, mean (SD; range)	71·8 (20·5; 19·0–122.0)
FEV1%pred category, no (%)	
<40%pred	14 (6·3)
40–70%pred	85 (38·1)
70–90%pred	75 (33·6)
90–110%pred	44 (19·7)
>110%pred	5 (2·3)
IV-treated PEx[b], no. (%)	
None	169 (75·8)
One or more	54 (24·2)
BMI in adults (kg/m^2) ≥ 18 years, mean (SD; range)	21·9 (2·7; 16·7–35·9)
BMI in adults (kg/m^2) ≥ 18 years, category, no (%)	
<18 kg/m^2	9 (5·1)
18–21 kg/m^2	60 (33·7)
21–24 kg/m^2	72 (40·4)
>24 kg/m^2	37 (20·8)
BMI Z-score in children 12-18 years, mean (SD; range)	-0·2 (0·9; -2·0–1·8)
BMI Z-score in children 12-18 years, category, no (%)	
<-1	9 (20·0)
-1 – +1	29 (64·4)
>1	7 (15·6)
Overall Q-Life score, median (IQR)	66·7 (50·0–87·5)
CFQ-R respiratory domain score, median (IQR)	72·2 (61·1–88·9)
Overall CFQ-R score[c], median (IQR)	77·2 (66·2–85·9)

Abbreviations: BMI: body mass index; CFTR: Cystic fibrosis transmembrane conductance regulator; CFQ-R: Cystic Fibrosis Questionnaire-Revised; FEV1%pred: forced expiratory volume in 1s percentage predicted; IV: intravenous; MF: minimal function; PEx: pulmonary exacerbations; RF: residual function.

[a] CFTR modulator treatment at the time of study enrollment.

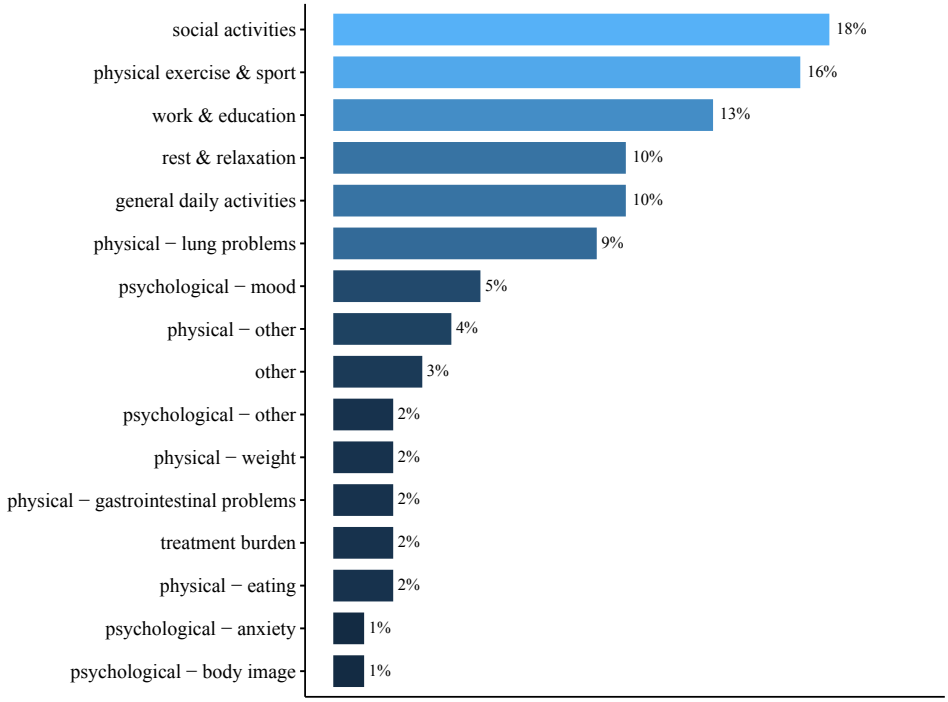
[b] IV-treated PEx in year prior to first study visit.

[c] The overall CFQ-R score was calculated by the mean of the twelve CFQ-R domain scores.

Individual quality of life items

Overall, 96 participants (43%) described three personal Q-Life items, whereas 65 participants (29%) and 62 participants (28%) reported four and five items, respectively. This resulted in a total of 858 self-described Q-Life items. As illustrated in **figure 2**, these items were most frequently labeled with the categories: social activities (n=150; 18%), physical exercise and sport (n=139; 16%), work and education (n=114; 13%), general daily activities (n=89; 10%), rest and relaxation (n=82; 10%) and physical – lung problems (n=78; 9%). Examples of self-described Q-Life items are provided for each category in **supplementary table 1**.

Figure 2. Distribution of categories selected to label self-described quality of life items



Study participants described a total of 858 personal Q-life items. These items had to be labeled with one of the 16 pre-defined categories that participants considered most appropriate.

Reliability

Internal consistency of individual Q-Life scores was high, based on Cronbach's α of 0·83 when at least three personal Q-Life items were described (n=223). Consistency was slightly higher when assessed in those who described at least four (n=127) or five items (n=62), with Cronbach's α of 0·87 and 0·90, respectively. The subgroup of the cross-sectional study (n=27) showed an excellent stability of overall Q-Life scores after 14 days (ICC: 0·90; 95% CI 0·65–0·92; p<0.001). This was consistent when assessed separately for the first three self-described items, according to ICCs of 0·73–0·82 (**table 2**).

Table 2. Test-retest reliability of Q-Life scores (n=27)

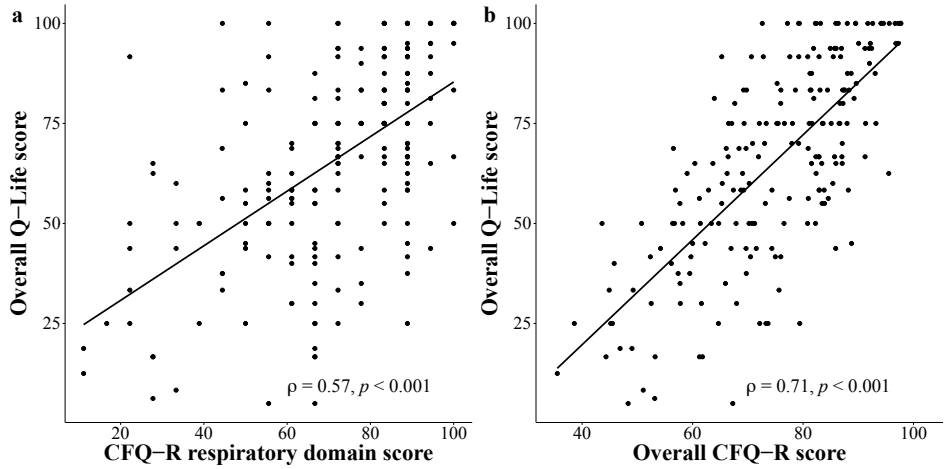
Q-Life scores	ICC[a]	95% CI	P-value
Overall score	0·90	0·78–0·96	<0·001
Item 1	0·73	0·49–0·86	<0·001
Item 2	0·81	0·62–0·91	<0·001
Item 3	0·73	0·48–0·87	<0·001

Abbreviations: ICC: intraclass correlation coefficient.
[a] ICCs were calculated for overall Q-Life scores and for the separate scores of the first three self-described items at baseline and after 14 days in clinically stable subgroup of participants.

Validity

Construct validity of Q-Life scores was assessed in multiple ways. First, overall Q-Life scores were associated with FEV1%pred (ρ=0·41, p<0·001), which indicates that participants with a better lung function reported higher Q-Life scores. In addition, overall Q-Life scores were positively associated with CFQ-R respiratory domain scores (ρ=0·57, p<0·001) and overall CFQ-R scores (ρ=0·71, p<0·001; **figure 3**). We did not observe a substantial impact of age or sex on the strength of these associations (**supplementary figure 2**). Furthermore, overall Q-Life scores were able to capture differences in CF disease severity, as pwCF who did not experience any IV-treated PEx had higher overall Q-Life scores compared to those who experienced at least one IV-treated PEx in the year prior to study participation (median difference: 16·3, 95% CI: 6·7–25·0, p<0·001). The association between overall Q-Life scores and IV-treated PEx is shown in **supplementary table 2** and **supplementary figure 3**. In addition, children with CF aged 12-18 years reported higher overall Q-Life scores than adults ≥18 years (median difference: 18·3, 95% CI: 10·0–25·0, p<0·001). Overall Q-Life scores did not significantly differ between females and males (difference in median: -1·8, 95% CI: -8·3–5·0, p=0·70). Similar characteristics were observed in our data for CFQ-R respiratory domain scores and overall CFQ-R scores (**supplementary table 3**).

Figure 3. Association of overall Q-Life scores with CFQ-R scores



Overall Q-Life scores were moderately associated with Cystic Fibrosis Questionnaire-Revised (CFQ-R) respiratory domain scores (a) and with overall CFQ-R scores, calculated by the mean of the twelve CFQ-R domain scores (b). ρ = Spearman's correlation coefficient (black line).

Sensitivity to detect change

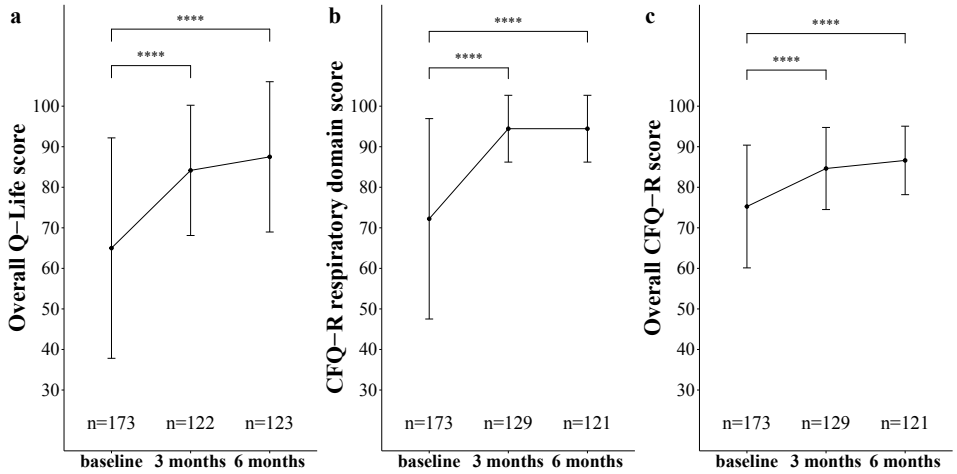
After 3 months of treatment with ETI, 122/173 (71%) of the participants completed a second Q-Life measurement remotely (**figure 1**), whereas 129/173 (75%) also completed the CFQ-R. In total, 145/173 (84%) of the participants returned to the clinical follow-up visit after 6 months of ETI. Of this subgroup, 123/145 participants completed a Q-Life measurement (**figure 1**) and 121/145 completed the CFQ-R, indicating that Q-Life and CFQ-R data were missing for 51/173 (29%) and 44/173 (25%) participants after 3 months of ETI, as well as for 50/173 (29%) and 51/173 (29%) participants after 6 months of treatment, respectively. **Supplementary table 4** shows a comparison of baseline characteristics between participants who did and did not complete a follow-up Q-Life measurement after 3 and 6 months.

Median overall Q-Life score improved from 65·0 (IQR:45·0–63·3) at baseline to 84·2 (IQR: 75·0–95·0) after 3 months and subsequently to 87·5 (IQR: 75·0–100·0) after 6 months, with a difference in median of 20·8 (95% CI 17·5–25·0, p<0·001 in paired samples; **figure 4a**). The magnitude of change was comparable to the change in CFQ-R respiratory domain score (difference in median: 22·2, 95% CI 19·4–25·0, p<0·001; **figure 4b**), which improved from 72·2 (IQR: 55·6–88·9) at baseline to 94·4 (IQR: 83·3–100·0) after 3 months and remained 94·4 (IQR: 83·3–100·0) after 6 months.

Both changes were considerably higher than the change in overall CFQ-R score, which increased from 75·3 (IQR: 65·3–85·5) at baseline to 84·6 (IQR: 77·4–90·9)

and 86·6 (IQR: 79·1–91·7), after 3 and 6 months, respectively (difference in median: 10·0, 95% CI 7·9–12·1, p<0·001; **figure 4c**). As illustrated in **figure 4**, the CFQ-R seemed to have reached a ceiling after 3 months of treatment with ETI. Q-Life scores demonstrated a comparable scale-responsiveness, but showed slightly lower absolute values after 3 and 6 months of treatment and a larger individual variance, suggesting that its ceiling may not have been reached. Median changes per self-described Q-Life item are summarized by category in **supplementary table 5.**

Figure 4. Sensitivity to detect change of overall Q-Life and CFQ-R scores



Median overall Q-Life scores significantly changed from baseline after 3 and 6 months of treatment with elexacaftor/tezacaftor/ivacaftor (a). The magnitude of change was comparable to the median change in CFQ-R respiratory domain scores (b), and higher than the median change in overall CFQ-R scores (c). Error bars represent median absolute deviation. Significance level p<.001 = ****.

DISCUSSION

The Q-Life app is a short personalized ePROM, developed in co-creation with patients and validated to capture quality of life on an individual level in 223 pwCF ranging in age from 12 to 58 years.

Reliability, validity and sensitivity to detect change of personal quality of life scores measured with the Q-Life app were good to excellent. These psychometric properties are at least comparable or slightly better than reported for the CFQ-R, which is the most widely used disease-specific PROM in CF [21,22]. The respiratory symptom subscale of the CFQ-R has been validated most extensively [19,23] and

is still the main focus of important CF-related clinical trials to demonstrate the impact of new treatments on quality of life [16,17,24–26].

Interestingly, however, our results illustrated that individuals with CF did not frequently consider respiratory symptoms as important or relevant to their quality of life, as personal Q-Life items related to lung problems were only described in 9% of total. Although respiratory symptoms are a hallmark of CF [15], these findings suggest that assessment of disease-specific symptoms may not be not sufficient to capture quality of life for most pwCF. Furthermore, it emphasizes the added value of a patient-centered personalized PROM like the Q-Life app, which is sensitive to track changes in other quality of life domains that are important and relevant for individuals.

Different types of PROMs require different validation approaches, which is acknowledged by regulatory authorities [2]. As personal Q-Life items are self-described by individual participants and not pre-defined, validation of the content of a personalized tool deviates from the regular validation process of standardized generic and disease-specific PROMs [2]. The Q-Life app intends to measure the same general construct of personal quality of life, but the content of the personal quality of life items is variable between participants and will inherently vary across different settings. This indicates that content validity can only be assessed on an individual level. Even though the content of individual items is derived directly from the participant, verification will be necessary to ensure content validity per individual and setting.

Internal consistency and test-retest reliability of Q-Life scores were high to excellent, even with a limited set of three to five self-described quality of life items with a content that varied per individual. These reliability measures were stronger than observed in the CFQ-R validation study [19]. Consistency seemed to increase with an increasing number of personal Q-Life items, although this might be influenced by the lower sample size of the groups who selected four and five personal items. In terms of consistency and relevance, three to five personal items seemed sufficient to capture all relevant aspects of quality of life for the majority of study participants, but the most optimal number of personal items might vary in different settings and should be further researched. Criterion validity and construct validity were demonstrated by associations of overall Q-Life scores with CFQ-R scores, which were regarded as reference standard to measure the concept quality of life in pwCF, as well as with measures of disease severity such as FEV1%pred, IV-treated PEx and age. The association and discriminative capacity of Q-Life scores and CFQ-R scores with measures of disease severity were comparable in our data and slightly

better than previously reported for the CFQ-R [19], substantiating validity of the Q-Life app. The association of Q-Life scores with FEV1%pred seems to be slightly lower than the association between the CFQ-R scores with FEV1%pred, although these correlations fall within the same range. These findings might be explained by the fact that Q-Life scores were only partly based on respiratory symptoms in 9% of participants, whereas respiratory symptoms have a more prominent role in the CFQ-R. This also supports the hypothesis that quality of life in general is only partly dependent on lung function or respiratory symptoms.

The ceiling effects at baseline may be explained by the liberal method of describing personal quality of life items, as participants were asked to describe items that were most important and relevant for their personal situation, which does not necessarily mean that these aspects are also affected by CF. As ceiling effects cause an increased skewness of the score distribution and subsequently an underestimation of the mean, we only used median scores in the analysis of this study. Ceiling effects generally reduce sensitivity to detect change, yet the cohort study showed that the Q-Life app was still sensitive to detect a group-level change in median overall Q-Life scores, at least when highly effective CFTR modulator therapy is initiated. The responsiveness of overall Q-Life scores was comparable to median CFQ-R respiratory domain scores and much higher than the changes in median overall CFQ-R scores in our data as well as in the non-respiratory CFQ-R domains in the phase 3 ETI trials [27]. Post-ETI, absolute median overall Q-Life scores were slightly lower than CFQ-R respiratory domain scores and individual variability was substantially higher. As illustrated by our data, sensitivity to detect change may diminish in pwCF who are becoming less symptomatic, e.g. in those already using highly effective CFTR modulators, but also in children or in those with mild disease manifestations. Further research is warranted to examine and compare the ceiling effects and sensitivity to detect change of the Q-Life app and CFQ-R in these specific CF populations.

Several socioeconomic and clinical factors are associated with health-related quality of life of adolescents and adults with CF. Physical symptoms including lung function decline and pulmonary exacerbations as well as mental symptoms such as anxiety and depression usually have the broadest impact [28,29]. The current treatment landscape of CF, however, has led to profound changes in life perspectives of pwCF who are eligible for highly effective CFTR modulator therapy, which is in contrast with the urgent unmet need for personalized therapies for those who carry rare CFTR mutations that cannot be treated with these modulators. Therefore, the heterogeneous nature of CF disease manifestations and advancing but disparate treatment options ask for a more flexible, patient-centered approach

to adequately capture the impact of CF disease, treatment modalities and healthcare on individuals.

The Q-Life app is a unique tool aimed to measure what really matters to individual patients, as it contains a short, easy to use personalized list of important and relevant items, which takes little time to be composed and scored. The personalized nature and relevance, efficiency, sensitivity and flexibility of the Q-Life app could provide advantages over the relatively large and burdensome set of questionnaires that are currently used in CF, but additional studies will be needed to assess whether the Q-Life app has the potential to replace at least some of these traditional questionnaires in the future. In addition, ePROMs have general advantages over paper-based PROMs in terms of feasibility, utility, accuracy, acceptance and response rates, and are more easily integrated into electronic research data capture systems and medical records [30]. In the cohort study, we observed similar response rates for the remote visit and the clinical visit, suggesting that remote monitoring could provide a suitable opportunity to maintain contact with individuals with CF who do not need to be frequently monitored in-hospital. The relatively limited time to complete a measurement in the Q-Life app might allow for more frequent data entry (e.g. weekly or bi-weekly), but the flexibility of the Q-Life app supports accommodation to the most optimal frequency in different settings such as trials or healthcare, and may also be tailored to individual preferences.

There are several important limitations to this study. In this observational study, we demonstrated the use of a patient-specific PROM to assess the impact of CF disease and highly effective CFTR modulator treatment on quality of life of individuals with CF. Additional studies are warranted for further development and external validation of this personalized ePROM in different CF populations including ethnically minoritized individuals, children and parents or caregivers, in different countries and settings such as clinical trials and healthcare, and in the context of e.g. different treatment modalities or life events. In addition, the minimal clinical important difference should be assessed in future studies [31]. Further research may also elucidate the value of a personalized ePROM such as the Q-Life app in other chronic diseases. The time period of this observational study was also an important limitation, as the largest part was conducted during the COVID-19 pandemic. Although all participants were explicitly instructed to score the impact of CF on their quality of life, we could not rule out that the COVID-19 pandemic, including the intermittent social distancing measures, may have had an impact on the study results. We were not able to include adolescents with CF in the panel of patient representatives who were involved in the development of the Q-Life app due to lack of availability, indicating that this part of the target population was

underrepresented in the initial development phase. In addition, not all members of the CF multidisciplinary team were involved in the core development team, indicating that potentially valuable input might have been missed. Furthermore, a follow-up measurement in the cohort study was missing in 29% of participants. This could have over- or underestimated the sensitivity to detect change of the Q-Life app, although it was still comparable to the CFQ-R respiratory domain scores. In conclusion, this first validation study showed that the Q-Life app is a reliable, valid and sensitive personalized ePROM to assess all aspects of quality of life that really matter to individuals with CF.

Acknowledgements
We are thankful to all Dutch people with CF and parents of children with CF who were involved in the development process of the Q-Life app; the people with CF across Europe who were involved in the development and testing of the international versions of the Q-Life app; and those who participated in this study and provided important and valuable feedback for further development and implementation. We also thank the Dutch Cystic Fibrosis Foundation and CF Europe for their support and collaboration in this project; Solviteers for the development and hosting of the Q-Life app; and the CF clinical research team of the Wilhelmina Children's Hospital and UMC Utrecht for their help with the organization and data collection during the cohort study.

Funding
This project received funding from the Dutch Cystic Fibrosis Foundation (CFOS 2019 and CFOS 2020) and Health Holland (project number TKI-LSH-DT2019-NCFS).

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Conflicts of interest

C.K. van der Ent reported a grant (CFOS 2022) from The Dutch Cystic Fibrosis Foundation (money to institution), outside of the submitted work. No other disclosures were reported.

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SUPPLEMENTARY **Methods.**

Q-Life app development

The Q-Life app was developed as a personalized electronic patient-reported outcome measure intended to assess quality of life on an individual level in people with Cystic Fibrosis (pwCF). The development team consisted of a panel of three adults with Cystic Fibrosis (CF) and two parents of children with CF who accepted the invitation of the Dutch Cystic Fibrosis Foundation (NCFS) to participate as patient representatives in this project. In addition, one coordinating clinical researcher, two physicians with CF expertise (one adult respiratory physician and one pediatrician) and two software developers were part of this core development team. To ensure a central role of pwCF in this project, one of the CF panel members was involved in the entire development process. The research coordinator of the NCFS had an advisory role throughout the development process.

In the first development phase, the impact of CF on individual quality of life and the desirable properties of an individualized measurement tool were discussed during a focus group meeting and an individual interview with all 5 panel members using a standardized template of questions. The focus group discussion and individual interviews led to the decision to create an app in which pwCF can enter self-described items they consider important and relevant for their personal quality of life in an open text field. The aim was to create an efficient and relevant tool, keeping the number of items as low as possible and including only those aspects that were considered important and relevant for someone's individual quality of life. The panel considered three to five items as sufficient for this purpose. The instructions about how to describe and formulate the personal Q-Life items were also derived from this focus group and from the individual interviews. Personal items were considered as the primary and most important aspect of the app. The categories that were used to label these self-described items were intended to have a supportive role, so they were solely created to facilitate standardization and validation against the Cystic Fibrosis Questionnaire-Revised (CFQ-R). For this reason, we used the domains of the CFQ-R as a starting point. During the focus group meeting, categories were extended and renamed to improve understanding of the categories, as it is important for users to understand the categories to be able to label personal items with a most appropriate one.

After the development of the Q-Life app, pre-testing was conducted with the five panel members. Based on one cycle of cognitive interviews, all categories were retained. The categories body image and treatment burden were renamed to improve understanding of this category. In addition, the instructions about how

to describe personal quality of life items were slightly modified. Furthermore, we added the instruction to complete the following sentence: "I find it important that I …", aimed to facilitate standardization of the self-described personal quality of life items. Survey length was not modified. Most testers indicated that the ranking of the personal items in order of importance was difficult or deemed irrelevant. Therefore, we decided to drop the ranking as a compulsory part of the app in these studies, but retained this as optional feature. All other modifications based on the pre-testing were related to the design and functioning of the app and aimed to improve clarity, user-friendliness and bug fixing. The first version of the app was also discussed with and tested by the CF multidisciplinary team, who provided additional input regarding the content, clarity and design of the app. After completing the second development phase, the app was considered ready for use.

SUPPLEMENTARY **Tables.**

Supplementary table 1. Examples of self-described quality of life items per category

Example – I find it important that I… [a]	Category
Can take care of myself, my animals and my household independently	General daily activities
Can work fulltime	Work and education
Can dance like the others, without getting extremely tired	Physical exercise and sport
Find a good balance between exertion and relaxation	Relaxation and rest
Have enough energy to spend time with friends	Social activities
Have few respiratory infections	Physical – lung problems
Experience few abdominal complaints, a calm bowel	Physical – gastrointestinal problems
Can enjoy food	Physical – eating
Maintain a stable weight	Physical – weight
Am in a more consistent shape, so I can rely on how much energy I'll have tomorrow	Physical – other
Feel happy	Psychological – mood
Learn to accept the uncertainty of the future	Psychological – anxiety
Maintain a positive image of my body	Psychological – body image
Can feel mentally at peace	Psychological – other
Have more time for other activities instead of having to undergo long intensive nebulization therapy	Treatment burden
Can participate in society	Other

[a] Examples of self-described quality of life items, labelled with categories that could be selected from a pre-defined list. Participants were instructed to describe their personal items by completing the following sentence: "I find it important that I…".

Supplementary table 2. Logistic regression model estimates of IV-treated PEx and overall Q-Life scores

	Odds ratio	95% confidence interval	p-value
Intercept	1·40	0·61–3·19	0·423
Overall Q-Life score	0·98	0·96–0·99	<0·001*

Abbreviations: IV: intravenous; PEx: pulmonary exacerbations.
Interpretation: the odds of IV-treated PEx decreased with increasing Q-Life scores.
*Significance level p<0·05.

Supplementary table 3. Construct validity of Q-Life scores compared to CFQ-R scores

	Q-Life scores (95% CI)	CFQ-R respiratory subdomain score (95% CI)	CFQ-R overall scores (95% CI)
Association with FEV1%pred	0·41***	0·49***	0·50***
Difference between groups with and without IV-treated PEx [a]	16.3 (6.7–25.0)***	16·7 (11·1–22·2)***	9·6 (5·1–14·3)***
Difference between children 12-18 years and adults >18 years [b]	18·3 (10·0–25·0)***	11·1 (5·6–16·7)***	8·3 (3·9–12·9)***
Difference between females and males	-1·8 (-8·3–5·0) [ns]	-5·6 (-11·1–0·0) [ns]	0·9 (-2·9–4·6) [ns]

Abbreviations: CFQ-R: Cystic Fibrosis Questionnaire-Revised; FEV1%pred: forced expiratory volume in 1s percent predicted; IV: intravenous; PEx: pulmonary exacerbations.
[a] Difference in median indicates higher quality of life scores in group without IV-treated PEx compared to group with at least 1 IV-treated PEx in year prior to first study visit.
[b] Difference in median indicates higher quality of life scores in children compared to adults.
*** Significance level p<0·001. ns= not significant.

Supplementary table 4. Comparison of baseline characteristics between subgroups with complete and missing Q-Life measurements after 3 and 6 months of treatment with elexacaftor/tezacaftor/ivacaftor (ETI)

Baseline characteristics	Follow-up Q-Life measurement after 3 months ETI		Follow-up Q-Life measurement after 6 months ETI	
	Complete (n=122)	Missing (n=51)	Complete (n=123)	Missing (n=50)
CFTR genotype, no (%)				
Homozygous F508del	97 (79·5)	39 (76·5)	100 (81·3)	36 (72·0)
F508del/MF	23 (18·9)	6 (11·7)	22 (17·9)	7 (14·0)
F508del/RF	-	2 (3·9)	-	2 (4·0)
F508del/gating	1 (0·8)	1 (2·0)	-	2 (4·0)
F508del/unknown	1 (0·8)	3 (5·9)	1 (0·8)	3 (6·0)
CFTR modulator treatment[a], no. (%)				
None	24 (19·7)	11 (21·5)	23 (18·7)	12 (24·0)
Ivacaftor	1 (0·8)	1 (2·0)	-	2 (4·0)
Lumacaftor/ivacaftor	37 (30·3)	18 (35·3)	39 (31·7)	16 (32·0)
Tezacaftor/ivacaftor	60 (49·2)	21 (41·2)	61 (49·6)	20 (40·0)
Sex, no. (%)				
Female	57 (46·7)	25 (49·0)	60 (48·8)	22 (44·0)
Male	65 (53·3)	26 (51·0)	63 (51·2)	28 (56·0)
Level of education, no. (%)				
None	2 (1·7)	1 (2·0)	2 (1·6)	1 (2·0)
Primary/elementary school	4 (3·3)	1 (2·0)	4 (3·3)	1 (2·0)
Preparatory secondary vocational school	12 (9·8)	10 (19·6)	12 (9·8)	10 (20·0)
Secondary vocational school	36 (29·5)	17 (33·3)	39 (31·7)	14 (28·0)
Secondary school	16 (13·1)	9 (17·6)	17 (13·8)	8 (16·0)
Higher professional education	33 (27·0)	7 (13·7)	34 (27·6)	6 (12·0)
University	17 (13·9)	5 (9·8)	14 (11·4)	8 (16·0)
Missing	2 (1·7)	1 (2·0)	1 (0·8)	2 (4·0)
Age (years), median (IQR)	24·0 (19·0–30·8)	24·0 (19·0–31·0)	24·0 (19·5–29·0)	23·0 (17·0–34·0)
FEV1%pred, mean (SD)	75·0 (20·3)	74·0 (17·6)	73·2 (20·1)	75·8 (18·4)
IV-treated PEx[b], no. (%)				
None	96 (78·7)	41 (80·4)	91 (74·0)	46 (92·0)
One or more	26 (21·3)	10 (19·6)	32 (26·0)	4 (8·0)
BMI in adults (kg/m^2) ≥ 18 years, mean (SD)	21·7 (2·4)	21·2 (2·5)	21·6 (2·3)	21·5 (2·7)
BMI Z-score in children 12-18 years, mean (SD)	-0·2 (1·0)	0·0 (0·7)	-0·2 (1·0)	-0·1 (0·8)
Q-Life score, median (IQR)	66·7 (50·0–83·3)	58·3 (42·7–75·0)	66·7 (47·5–83·3)	59·1 (43·8–91·3)
CFQ-R respiratory domain score, median (IQR)	72·2 (59·7–88·9)	72·2 (50·0–83·3)	72·2 (55·6–83·3)	72·2 (55·6–88·9)
Overall CFQ-R score[c], median (IQR)	77·3 (65·3–85·7)	73·3 (63·7–83·9)	75·3 (64·9–85·0)	74·4 (65·7–86·2)

Abbreviations: BMI: body mass index; CFTR: Cystic fibrosis transmembrane conductance regulator; CFQ-R: Cystic Fibrosis Questionnaire-Revised; ETI: elexacaftor/tezacaftor/ivacaftor; FEV1%pred: forced expiratory volume in 1s percentage predicted; IV: intravenous; MF: minimal function; PEx: pulmonary exacerbations; RF: residual function.

[a] CFTR modulator treatment at the time of study enrollment.

[b] IV-treated PEx in year prior to first study visit.

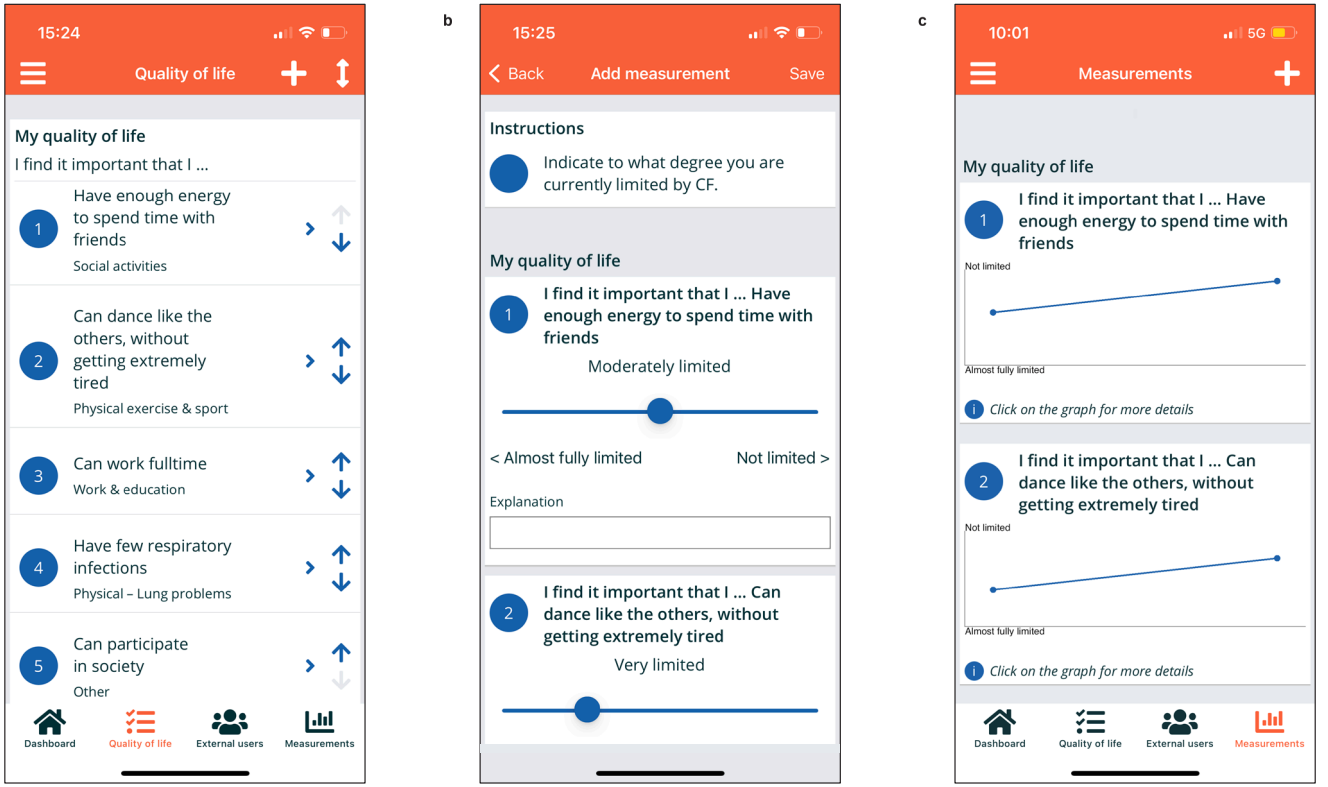
[c] The overall CFQ-R score was calculated by the mean of the twelve CFQ-R domain scores.

Supplementary table 5. Q-Life scores per self-described item grouped by category

Category	Q-Life scores at baseline		Q-Life scores after 6 months ETI	
	n	Median (IQR)	n	Median (IQR)
General daily activities	68	4·0 (3·0–5·0)	45	4·0 (4·0–5·0)
Work and education	91	4·0 (3·0–5·0)	68	5·0 (4·0–5·0)
Physical exercise and sport	112	4·0 (3·0–4·0)	82	5·0 (4·0–5·0)
Relaxation and rest	65	4·0 (3·0–5·0)	43	5·0 (4·0–5·0)
Social activities	116	4·0 (3·0–5·0)	83	5·0 (4·0–5·0)
Physical – lung problems	65	3·0 (2·0–4·0)	47	5·0 (4·0–5·0)
Physical – gastrointestinal problems	16	3·0 (2·0–3·0)	12	3·5 (3·0–4·0)
Physical – eating	19	3·0 (3·0–4·0)	13	4·0 (3·0–5·0)
Physical – weight	12	3·0 (2·0–3·3)	6	3·5 (3·0–4·8)
Physical – other	27	3·0 (3·0–4·0)	16	4·0 (4·0–5·0)
Psychological – mood	33	3·0 (3·0–4·0)	24	4·0 (2·0–4·3)
Psychological – anxiety	7	2·0 (2·0–2·5)	6	3·5 (2·3–4·0)
Psychological – body image	11	3·0 (2·0–4·0)	8	4·0 (3·0–4·0)
Psychological – other	9	3·0 (1·0–5·0)	9	5·0 (3·0–5·0)
Treatment burden	12	3·0 (3·0–4·0)	10	5·0 (4·0–5·0)
Other	14	3·5 (2·3–4·8)	9	4·0 (3·0–5·0)

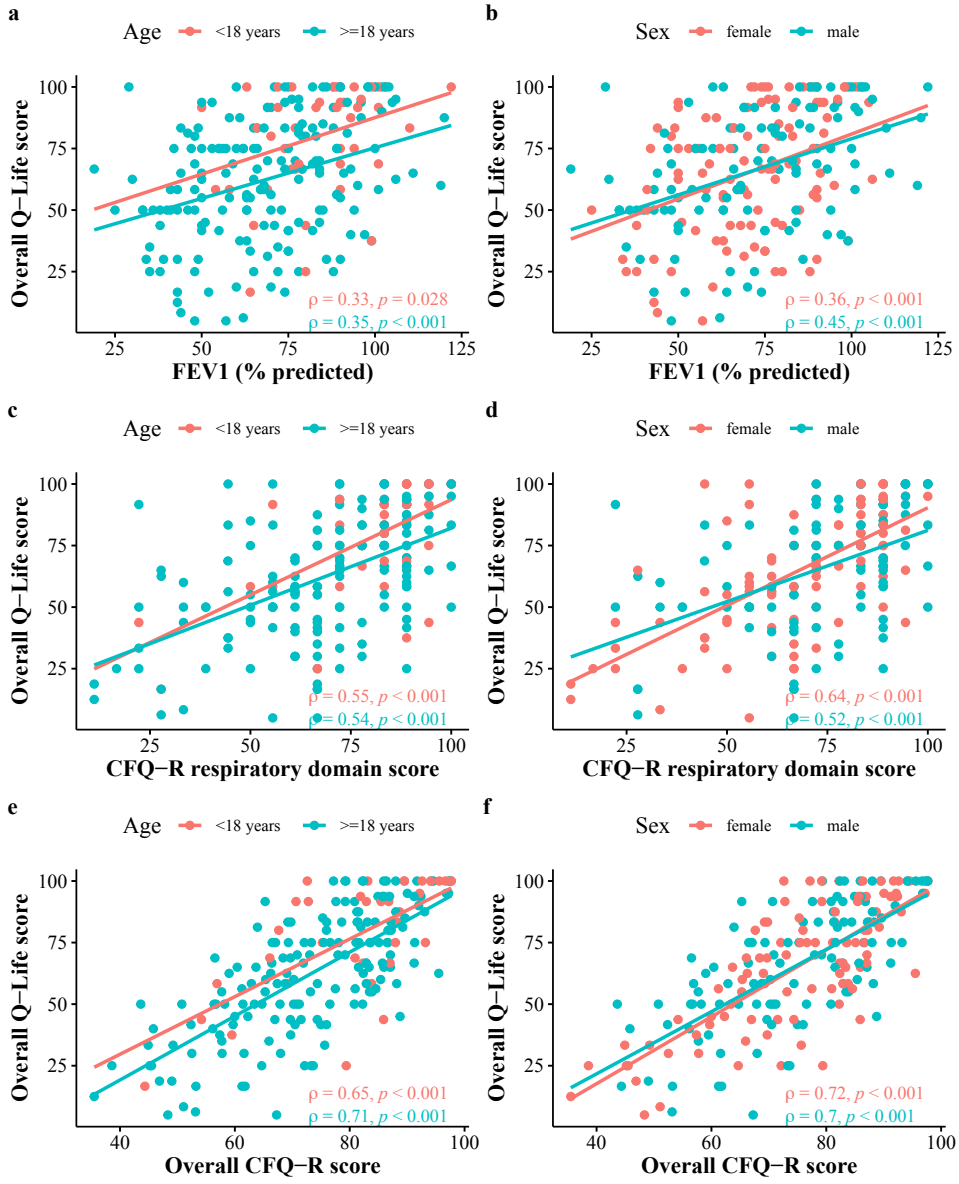
Abbreviations: ETI: elexacaftor/tezacaftor/ivacaftor; n: number of self-described items in cohort study for which a score was completed at baseline and after 6 months of treatment with ETI.

Supplementary figure 1. Overview of Q-Life app



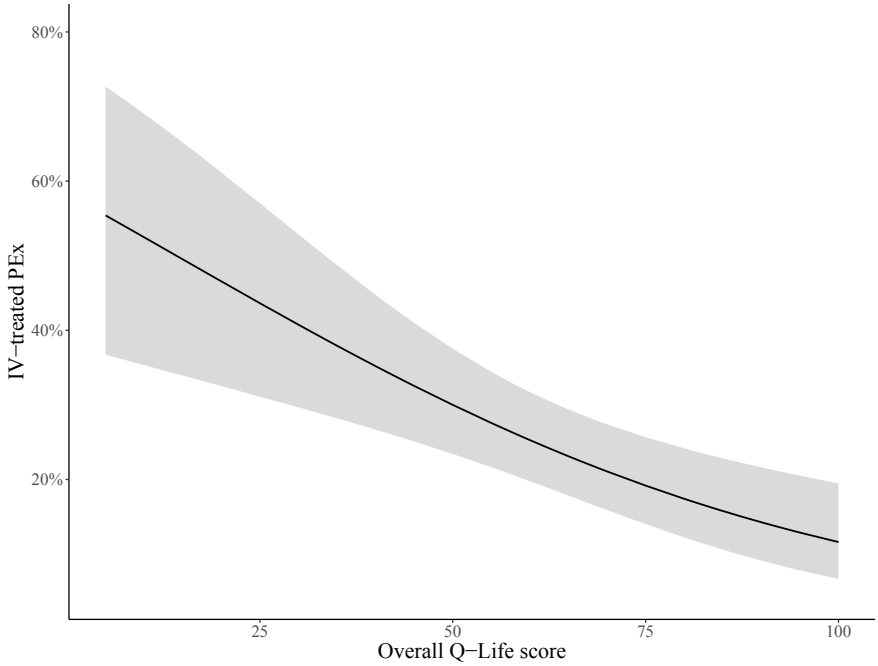
a) Step 1: example of a personal set of self-described Q-Life items, labelled with the most appropriate category. Personal items can be ranked by using the arrows on the right. b) Step 2: for each item, users can score to what degree they currently feel limited by CF on a 5-point Likert scale: almost fully limited (1 point), very limited (2 points), moderately limited (3 points), minimally limited (4 points) or not limited (5 points). c) Graphical display of results.

Supplementary figure 2. Associations of overall Q-Life scores with FEV1%pred and CFQ-R scores in subgroups



The strength of the associations between overall Q-Life scores and FEV1%pred, CFQ-R respiratory domain scores and overall CFQ-R scores did not substantially differ between age groups (a,c,e) or between females and males (b,d,f). ρ = Spearman's correlation coefficient.

Supplementary figure 3. Association of IV-treated PEx in the year prior to study participation with overall Q-Life scores



The probability of experiencing a pulmonary exacerbation treated with intravenous antibiotics (IV-treated PEx) decreases with an increasing Q-Life score. The black line represents the average model estimates derived from **supplementary table 2**. The grey ribbon represents the 95% confidence interval.

CHAPTER 7

General discussion

MAIN CONCLUSIONS **of the research described in this thesis**

1. CFTR modulators have marked the beginning of a new era for people with Cystic Fibrosis who are eligible for these disease-modifying therapies, which reduce clinical manifestations and prevent disease progression. In search for an effective treatment for the remaining people with CF who are not eligible for CFTR modulator therapy, several new agents have entered the clinical drug development pipeline, including read-through agents, mRNA- and gene therapy (**chapter 2**).

2. CFTR protein function quantified by the forskolin-induced intestinal organoid swelling (FIS) assay is associated with long-term CF disease progression, including FEV1 decline and the odds of developing CF-related co-morbidities pancreatic insufficiency, CF-related liver disease and CF-related diabetes (**chapter 3**).

3. In contrast with FIS, sweat chloride concentration (SwCl) was not associated with long-term CF disease progression, suggesting that FIS performs better as a prognostic CFTR-function biomarker than SwCl (**chapter 3**).

4. Long-term real-world effectiveness of dual CFTR modulators in people with CF homozygous for F508del is less strong than the efficacy reported in clinical trials and varies considerably between individuals with CF and different baseline FEV1 levels (**chapter 4**).

5. Long-term effectiveness of dual CFTR modulator therapy currently remains difficult to predict on an individual level, as we could not identify clinical or biological predictors of long-term change in FEV1 decline after CFTR modulator initiation (**chapter 5**).

6. The Cystic Fibrosis Questionnaire-Revised respiratory domain score is currently the reference standard to assess disease-specific quality of life in CF-related clinical trials, but only 9% of people with CF consider respiratory symptoms as important or relevant to their personal quality of life (**chapter 6**).

7. The Q-Life app is a reliable, valid and sensitive personalized electronic patient-reported outcome measure that can assess all aspects that really matter for quality of life of individuals with CF (**chapter 6**).

DISCUSSION

The latest highly effective cystic fibrosis transmembrane conductance regulator (CFTR) modulator elexacaftor/tezacaftor/ivacaftor (ETI) has been a gamechanger in the field of Cystic Fibrosis (CF) therapies, changing the lives and future perspectives of ~70-80% of people with Cystic Fibrosis (pwCF) who are eligible for this triple CFTR modulator combination [1–3].

It may be clear that global research efforts should be prioritized to the development of effective new treatments for the ~20-30% of people with CF (pwCF) carrying mutations that cannot be rescued with CFTR modulators or (ultra-)rare mutations of which responsiveness to CFTR modulators is unknown [**chapter 2**]. Notwithstanding, additional work remains needed in pwCF who are eligible for CFTR modulator therapy, as individual treatment responses are heterogeneous and high costs are limiting access to these drugs across the globe. Furthermore, several CF-related complications may be partly reversible or irreversible and new long-term complications may emerge related to the treatment or to prolonged survival and aging.

The increasing heterogeneity of the changing CF population poses additional challenges to the development of new drugs and the translation of the impact of such therapies into real-world practice. Traditional short-term clinical endpoints that are commonly being used in CF-related drug trials may not be sensitive enough to demonstrate efficacy in the relatively small group of pwCF carrying rare or ultra-rare mutations. Consequently, new therapies that may have a limited or moderate measurable effect may not pass the clinical drug development pipeline, even though the impact may still be clinically significant for individuals. Furthermore, current clinical endpoints may lack sensitivity and relevance in pwCF already using ETI, who may only be willing to participate in trials that do not require discontinuation of their current CFTR modulating therapy. In addition, the ability to predict effectiveness is limited with traditional clinical endpoints, which is important for clinical decision-making in a real-world setting.

Long-term real-world data could play an essential role in the identification and validation of novel alternative (short-term) endpoints to guide clinical drug development and individual-level predictions in the contemporary CF population. This chapter discusses the utility of long-term real-world data for the development of new biomarkers such as patient-derived intestinal organoids or novel patient-reported outcome measures, leading to recommendations that can help to move forward towards a cure for all pwCF.

I. UTILITY OF LONG-TERM REAL-WORLD DATA

To investigate whether new drugs generate a meaningful impact on patients, clinical trials should include endpoints that are both relevant and sensitive to detect change in the target population. In progressive diseases like CF, endpoints are generally deemed relevant when related to either long-term outcomes such as survival and disease progression, or to quality of life. The main goal of clinical trials is to demonstrate drug safety and efficacy on a group level but not necessarily on an individual level, as the need to identify individual responders and non-responders to treatment is not a general requirement in trials. This is in contrast with real-world practice, in which decisions generally have to be tailored to individual patients. Consequently, appropriate endpoints that have the ability to capture short-term treatment effects that also translate into relevant long-term benefits on an individual level are not only essential to guide treatment decisions in a real-world setting, but can also inform e.g. reimbursement decisions by policy makers and insurance companies. This is especially relevant for expensive disease-modifying drugs such as CFTR modulators, which incur very high costs while individual treatment effects are heterogeneous. Yet establishing such a relationship between short-term trial outcomes and individual long-term real-world outcomes can be challenging, as it depends on a delicate interplay of variability in disease characteristics, measurement properties of the selected endpoints as well as the effect size of a therapeutic intervention.

This section will evaluate how main short- and long-term pulmonary outcomes in clinical trials are related to each other in the context of dual CFTR modulator therapy and how these trial outcomes translate into short- and long-term real-world outcomes on a group- and individual level. Subsequently, the use of long-term real-world data to identify and validate alternative endpoints and to innovate clinical trials will be delineated.

Relationship of clinical trial and real-world pulmonary outcomes in the context of dual CFTR modulators

Pulmonary outcomes such as forced expiratory volume in 1 s (FEV1) and pulmonary exacerbation rate (PEx) are the most commonly used primary or key secondary endpoints in CF-related clinical trials, due to the association with long-term disease progression and survival [4–10]. Nevertheless, both endpoints have their own restrictions in terms of feasibility and sensitivity, which may be partly related to intrinsic measurement variability, heterogeneity of disease symptoms and magnitude of treatment response.

The dual CFTR modulators lumacaftor/ivacaftor (LUM/IVA) and tezacaftor/ivacaftor (TEZ/IVA) have formed the starting point of targeted therapy for pwCF homozygous for the most frequent allelic variant F508del [11,12], and laid the foundation for further development of next-generation triple therapy elexacaftor/tezacaftor/ivacaftor (ETI) for pwCF carrying at least one F508del mutation [13–15]. Yet these first-generation dual CFTR modulator trials with moderate overall effect sizes also illustrate the difficulties with extrapolating short-term trial outcomes into individual long-term real-world outcomes, not at least due to the impact of non-CFTR dependent biology. This further contributes to heterogeneity in disease progression in an organ-specific manner and especially within the pulmonary domain where the impact of non-CFTR dependent factors on disease severity is relatively large.

Short-term vs. long-term trial outcomes

Using FEV1 as primary endpoint, international multi-center registration trials with dual CFTR modulators were sufficiently powered (n= 1106 LUM/IVA; n=504 TEZ/IVA) to establish a moderate short-term group-level efficacy in a selected population of pwCF homozygous for F508del with a baseline FEV1 between 40-90% aged 12 years and older [11,12]. Furthermore, a moderate reduction in PEx rate was captured within the 24-week study period, including those PEx leading to hospitalization or intravenous (IV) antibiotic treatment. In combination with the highly variable individual treatment responses reported in phase 2 and phase 3 trials [11,12,16,17], these findings elicited a widespread debate about the clinical relevance of such short-term changes that were deemed modest. In contrast with the FDA, EMA's Committee for Orphan Medicinal Products did not recognize an improvement of ≤4% in FEV1 as clinically relevant and did not confirm the orphan status for LUM/IVA at the time of marketing authorization [3]. Furthermore, the unfavorable cost-effectiveness profile of dual CFTR modulators has led to delayed or limited access to these drugs throughout many countries.

In contrast, the 96-weeks open-label extension trials constructed a more positive perspective on the long-term impact of dual CFTR modulators on disease progression, considering the relative reduction in annual FEV1 decline of 42-61% in treated participants compared to historical controls [18,19]. Furthermore, lower PEx rates reported in short-term trials were sustained, including those requiring hospital admission or IV antibiotics [18,19]. As change from baseline FEV1 is less sensitive over a long-term period due to the progressive nature of CF, these trials used change in FEV1 decline as efficacy endpoint. The absence of a control arm in such long-term studies increases trial efficiency but may also lead to biased results. The use of real-world data from matched historical controls may help to overcome

this problem, although trial outcomes might still be overestimated due to potential (unmeasured) differences in patient characteristics, received standard care or treatment adherence, in discordant time periods and different settings.

To summarize, efficacy of dual CFTR modulators is not only heterogeneous on the individual level, but also varies on a population-level between different endpoints, even across endpoints related to the respiratory system. Furthermore, short- and long-term changes may be independent, as short-term FEV1 improvement was substantially lower than in prior IVA trials in pwCF heterozygous for a gating mutation [20,21], whereas the magnitude of change in FEV1 decline was similar to the reported 47% reduction for IVA [22], which suggests a stronger long-term impact. In contrast, the reduction in PEx rate was more consistent across short- and long-term trials [11,12,18,19]. Taken together, these findings show that short-term trial results using traditional pulmonary endpoints are not always consistent with long-term trial results, at least with dual CFTR modulators that are considered moderately effective. Additional comparisons between short- and long-term trials and real-world studies in large and diverse cohorts will help to further identify how pulmonary outcomes in clinical trials translate into a real-world setting, which may further elucidate the actual impact on individual patients.

Short-term real-world vs. trial outcomes

Generalizability of randomized controlled trial (RCT) results to a real-world setting is usually hampered by the strict selection criteria and monitoring procedures in clinical trials, although the methodological data acquisition might be more consistent as compared to real-world settings. In this thesis, short- and long-term effectiveness of dual CFTR modulators were assessed in pwCF homozygous for F508del in a national real-world cohort using Dutch CF Registry data [**chapter 4**] and in a single center cohort study using routinely collected clinical data retrieved from electronic medical records [**chapter 5**]. Due to the relatively rapid successive development and market entry of LUM/IVA, TEZ/IVA and ETI, long-term follow-up of dual CFTR modulators beyond the first year of treatment has remained limited in a real-world setting. The studies described in this thesis were the first to report on the combined long-term effectiveness of dual CFTR modulators up to 3 years post-initiation [**chapter 4, chapter 5**].

This thesis showed that short- and long-term effectiveness of dual CFTR modulators in pwCF homozygous for F508del is less strong than efficacy reported in clinical trials [**chapter 4, chapter 5**]. Within the pulmonary domain, several observational real-world studies have reported short-term improvements in FEV1 ranging from 1.5 to 2.7% upon the first year after dual CFTR modulator initiation in pwCF

homozygous for F508del aged 12 years and older [**chapter 4**];[23–27], with the exception of one small study (n=36) showing a slightly larger increase of 5% after 6 months of LUM/IVA treatment [28]. Some other real-world studies that failed to detect statistically significant group-level changes in FEV1 [29–32] were conducted in small populations [29,30,32], included both adults and pediatric patients of 6 years and older [31] or only adolescents between 12-18 years [29].

As PEx are extremely difficult to capture in a real-world setting due to the lack of uniform and feasible criteria that justify the heterogeneity of PEx-related symptoms and subsequent treatment decisions in daily practice, the annual number of days receiving IV antibiotic treatment has been adopted as proxy for severe PEx. In line with RCT results, **chapter 4** reported a reduction of 28% in the number of days/year receiving IV antibiotics in the first year of dual CFTR modulator treatment compared to the year preceding CFTR modulator treatment. This was consistent with another study reporting changes in the number of PEx and in both oral and IV antibiotic courses in pwCF with severe lung disease [25]. Interestingly, another relatively large real-world cohort study (n=845) reported a comparable 35% reduction of the number and duration of IV antibiotic courses in pwCF who had continued treatment for 1 year, but not in those who discontinued treatment temporarily or permanently [23]. As for FEV1, some other studies failed to detect a significant short-term change in PEx in smaller [28] or different [31] real-world populations up to 1 year after treatment initiation. Despite the difference in sample size and characteristics of the study populations, these results suggest that real-world short-term effects in FEV1 are approximately half of the changes reported in dual CFTR modulator trials, whereas PEx rates are more consistent across both settings. This would support a further prioritization of PEx rates in clinical trials as indicators of treatment effect, at least in CFTR modulator-naïve pwCF.

Long-term real-world vs. trial outcomes

In **chapter 4**, average FEV1 decline improved from -1.4% per year to -0.5% per year in the years after dual CFTR modulator initiation. The pre-modulator decline of -1.4% per year was markedly lower than the -2.1% to -2.3% per year reported for the historical control groups used in long-term trials [18,19]. This difference may be related to a longer pre-modulator follow-up time up to 7 years in **chapter 4** which may have extended into childhood, wherein average rate of FEV1 decline is generally lower than in adolescence and early adulthood [33]. Nevertheless, the observed improvement of FEV1 decline after dual CFTR modulator initiation still reflected a relative change of 65% that compares to the trial data. In addition, **Chapter 5** reported a representative average FEV1 decline of -2.1% per year over the last 3 years pre-modulator treatment. Surprisingly, however, a group-level

improvement in long-term FEV1 decline was not observed in this study. This discrepancy is difficult to explain, but one may speculate that it could be related to the nature of the data, or to selection bias and incomparability of the study populations. In **chapter 4** (n=401), for instance, registry data was used containing only one annual best FEV1 measurement per year, whereas **chapter 5** included all FEV1 measurements within the follow-up period, which consists of on average 4 measurements per year (n=97). Consequently, the within-subject variability of FEV1 captured by repeated measurements might have been larger in the latter study and could have been negatively influenced by e.g. pulmonary exacerbations, differences in treatment adherence or use of co-medication, although a statistically significant association between IV treated PEx and the change in FEV1 decline could not be found. Furthermore, the study population in **chapter 5** only included pwCF of whom intestinal organoids were available. It could be possible that pwCF who were willing to undergo a rectal biopsy procedure have a more severe disease course.

Despite the short-term improvement in the first year of dual CFTR modulator initiation, our data suggested that the average annual duration of IV antibiotic treatment gradually increased again in the subsequent years, following the same trend as before CFTR modulator use [**chapter 4**]. This could be related to a decrease in adherence to e.g. antibiotic maintenance therapy, other co-medication or to the CFTR modulator itself, in line with the reported short-term differences in PEx between groups with continuous, intermittent or permanent discontinuation of dual CFTR modulators [23]. Other studies that compared the absolute number of PEx as well as oral and IV antibiotic courses over a 2- or 3-year period before and after dual CFTR modulator initiation did not detect a significant difference [**chapter 5**];[29].

In conclusion, short- and long-term effectiveness of dual CFTR modulators in real-life is less pronounced than the efficacy reported in clinical trials. Beyond the differences in study populations, designs and analysis methods, response profiles to dual CFTR modulators vary across clinically relevant pulmonary endpoints on a group-level and show high individual variability in both real-world and trial settings. As such, it remains needed to further explore how individual effects can be accurately measured and predicted, as to maximize the individual benefits over side effects for these life-long treatments.

Factors that underly the limited predictability of CFTR modulator effects

Ultimately, prediction of individual long-term treatment responses may help to guide treatment decisions and enable precision medicine, yet this remains difficult using

pulmonary outcome measures within pwCF carrying identical genotypes treated with a dual CFTR modulator [**Chapter 5**]. So far, several studies that focused on the predictive potential of different biomarkers and clinical characteristics could not identify predictors of either short- or long-term improvement in FEV1 after dual CFTR modulator initiation in pwCF homozygous for F508del [**chapter 5**];[24,28,30] or heterozygous for A455E [34]. The follow-up period of these studies ranged from 8 weeks to 3 years after CFTR modulator initiation, including up to 97 participants in **chapter 5**. Some but not all detected a moderate statistically significant group-level effect of dual CFTR modulators on different clinical outcomes.

Interestingly, several other studies demonstrated firm associations of CFTR function biomarkers with short-term clinical response to different types of CFTR modulators in pwCF carrying a variety of CFTR mutations [35,36]. This difference is likely explained by the relatively broad bandwidth of variation in the studied candidate predictors and outcome measures, in combination with the variation of disease characteristics within the study population and the variation in treatment effects of different CFTR modulators. The latter studies included participants with varying disease severity and tested different CFTR modulators that sorted a wider range of treatment effects, exceeding natural measurement variability in the outcomes and studied predictors.

Together, these studies suggest that the ability to detect individual-level associations between potential predictors and outcomes are likely dependent on I) the range in genetic and clinical characteristics of the study population and II) the range in effect size of the drug(s), relative to III) the intrinsic measurement variability of the outcome and predictors and IV) the impact of other (unmeasured) sources of variation on the outcome and predictors such as co-medication and treatment adherence, but also environmental and stochastic factors which contribute to ~50% of variation in FEV1 [37].

Given these results, would it be realistic to aim for individual predictions of treatment response in future studies, using traditional outcomes such as FEV1 or PEx, or should focus be shifted towards other alternative endpoints? And what would be the best approach to accomplish that? Ideally, the study population should be representative of the entire heterogeneous CF population, including pwCF in different disease stages, carrying different CFTR mutations and using different drugs. So far, however, all CFTR modulator trial populations have been pre-selected based on positive outcomes of *in vitro* cell models [38]. Subsequently, real-world studies have been restricted to subpopulations for whom specific CFTR modulator treatment became available, especially in long-term studies.

Furthermore, a combination of multiple predictors will probably be required to develop and validate an accurate prediction model. The amount of candidate predictors that can reliably be included is limited by the sample size. This indicates that large sample sizes would be required for traditional hypothesis-driven prediction models, which is challenging in rare diseases such as CF. In addition, not all true predictors may be measurable, at least not in a routine clinical setting. Alternative (data-driven) approaches based on e.g. machine learning or artificial intelligence algorithms may better be able to deal with high dimensional data, allowing the inclusion of many different data sources, extending beyond clinical data.

Predicting long-term FEV1 decline solely based on baseline characteristics before treatment initiation may remain difficult, but the high conditional R^2 reported in **chapter 5** suggests that a dynamic prediction model relying on previous FEV1 measurements might be an option to accurately predict long-term FEV1 decline after a short treatment trial. This requires at least a few repeated FEV1 measurements in the first weeks to months after treatment initiation to allow for a dynamic estimation of a change in FEV1 trend. Furthermore, focus might also be shifted towards prediction of alternative outcomes or combinations of outcomes such as composite or multivariate endpoints, which may better reflect treatment benefit compared to single outcome measures and may be more 'future proof' than traditional lung function measurements.

The utility of long-term real-world data in the contemporary CF population

What do these findings related to dual CFTR modulator treatment imply for the contemporary CF population, and what role can long-term real-world data play in future trials and clinical practice?

The CF population is becoming increasingly heterogeneous as a result of the next-generation triple therapy ETI, at least in countries where this highly effective therapy is available. Recent trials have shown a profound short-term improvement after 24-weeks of ETI in pwCF carrying at least one F508del mutation [13–15]. The treatment effects were sustained throughout 48 weeks of treatment [39] and seemed to stabilize pulmonary disease progression [40]. Consequently, pwCF who are being treated with ETI are having milder and more stable disease symptoms, including a higher average lung function with little to no decline and fewer pulmonary complications such as PEx and related hospitalizations.

Nevertheless, response to ETI is still highly variable between individuals and the impact of reversible and irreversible CF-related co-morbidities may differ across individuals upon CFTR modulation. Therefore, pwCF with limited or low responses

to ETI may still benefit from additional treatments, even when the effects are moderate, as observed for dual CFTR modulator treatment. In addition, this is even more important for the unfortunate minority of ~20-30% pwCF carrying CFTR modulator-unresponsive mutations with severe disease manifestations or rare mutations of which responsiveness is unclear. Consequently, prediction of individual long-term treatment response remains relevant to select individuals for additional or different new treatments, to inform on continuation or cessation of other (maintenance) therapies, or to support decision-making regarding frequency and setting of follow-up (e.g. remote or hospital visits).

Because traditional pulmonary endpoints may become even less sensitive and relevant in the current CF population, existing long-term real-world data may also be used to identify new predictors of long-term treatment response and/or disease activity, which could eventually serve as novel alternative or surrogate endpoints in future clinical trials.

Moreover, long-term real-world data can be integrated in clinical trials as external comparator arm to increase operational efficiency of trials, as exemplified by the open-label dual CFTR modulator extension trials. Additionally, long-term real-world data may even reshape the landscape of future clinical trials, which will become even more efficient when real-world data can be fully integrated in trials at multiple levels [41]. This will, however, not only require high quality data, but also novel technologies that can facilitate this transformation. Although CF registries encompass very relevant quality-controlled data, such technologies will also enable us to draw upon a vast number of data sources beyond regular clinical data, including but not limited to data derived from wearables and biological data.

In conclusion, moderate improvements in traditional pulmonary outcomes after dual CFTR modulator therapy substantially differ across short- and long-term studies in trial and real-world settings. Overall, long-term impact appeared stronger than short-term benefits, although effectiveness is less pronounced than the efficacy reported in trials. Individual predictions of moderate but potentially relevant long-term benefits of new disease-modifying treatments remain important to identify and select potential responders and support clinical-decision making in real-world practice. Yet prediction of pulmonary outcomes stays difficult due to the relatively limited impact of a moderate treatment effect in pre-selected populations compared to the large impact of intrinsic measurement variability and other non-CFTR dependent sources of variation. In contrast with clinical outcomes, CFTR function biomarkers such as the intestinal organoid model may quantify the individual impact of CFTR modulators drugs more precisely, potentially leading

to more accurate clinical effect size estimations. The next paragraph will discuss the predictive potential of the intestinal organoid model and the required steps to utilize this model as an alternative surrogate endpoint in clinical trials.

II. THE INTESTINAL ORGANOID MODEL AS SURROGATE ENDPOINT IN FUTURE CLINICAL TRIALS

Biomarkers can have a rich array of pre-clinical and clinical applications, as they help to fast-track basic science and inform drug discovery, early drug development, dose-selection and clinical trial design, for instance by guiding patient stratification or inclusion as surrogate endpoint [42]. Currently accepted endpoints and required sample sizes in traditional trial designs may no longer be feasible to demonstrate efficacy of new drugs or non-inferiority to highly effective CFTR modulators within the changing CF population, including adults who are becoming less symptomatic and children. Biomarkers of CFTR function may therefore be increasingly relevant for late-stage clinical development of compounds aiming to correct the basic CFTR protein defect [43]. Yet to serve as surrogate outcomes in registration trials, biomarkers require validation and acceptation by regulatory agencies, which depend on the predictive value for disease severity, natural disease progression and responsiveness to treatment [44].

This section discusses the potential role of intestinal organoids as CFTR function biomarker in the context of CF diagnosis, prognosis and treatment response. Subsequently, intestinal organoid assays will be compared to other CFTR function biomarkers and the next steps towards validation of the organoid model as a surrogate outcome in clinical trials will be outlined.

Excellent diagnostic accuracy of intestinal organoids to discriminate between CF and healthy individuals

Intestinal organoids generated out of rectal biopsies of pwCF exhibit a distinct morphology than organoids derived from healthy individuals. Compared to the large and round fluid-filled organoid lumina of healthy people, the organoids of pwCF appear smaller and denser with limited lumen formation, reflecting the diminished epithelial ion- and water transport into the organoid lumen. The steady-state lumen area (SLA) assay, which quantifies the lumen area as a percentage of the total organoid size, was able to accurately discriminate between non-CF organoids expressing high CFTR function and CF organoids with little to no CFTR function [35]. In addition, the SLA showed differences within CF organoids derived from individuals with class I-III mutations, which generally exhibit a more

severe disease severity, compared to individual with class IV-V mutations, which are known to express relatively milder disease symptoms due to residual CFTR protein function [35].

More recently, a semi-automated method to quantify organoid morphology based on roundness and fluorescent intensity differences between organoid lumen and the apical membrane (ROMA assay) showed a 100% sensitivity and specificity in discriminating organoids from pwCF and healthy individuals [45].

To summarize, these studies showed an excellent diagnostic accuracy of intestinal organoid assays to discriminate between CF and healthy controls, as these individuals exhibit relatively large differences in CFTR function at both ends of the measurement spectrum. Future studies should focus on the added diagnostic value in the context of difficult-to-diagnose CF within currently established diagnostic guidelines, such as in individuals carrying one or two rare uncharacterized CFTR mutations and an inconclusive sweat test.

Intestinal organoids as a prognostic biomarker of long-term CF disease progression

After the first pioneering research describing the development of the forskolin-induced swelling (FIS) assay for quantification of CFTR function in intestinal organoids of pwCF [46], two relatively small cross-sectional proof-of-concept studies supported that residual CFTR function quantified by FIS was associated with multiple clinical outcomes in infants carrying a variety of CFTR mutations and in adults homozygous for F508del [47,48].

In addition, the longitudinal study in **chapter 3** has been the first to establish individual-level associations of FIS with long-term CF disease progression. Results showed that residual CFTR function quantified by FIS of patient-derived cystic fibrosis organoids was associated with long-term annual FEV1 decline and odds of developing CF-related co-morbidities pancreatic insufficiency, CF-related liver disease and CF-related diabetes. This study comprised a large population consisting of 173 individuals with CF of varying ages, carrying many distinct CFTR mutations. Notably, results were adjusted for important confounders such as age and genotype, which further strengthen these findings.

Together, these studies suggested that CFTR function measurements in intestinal organoids hold great potential as a prognostic biomarker, based on individual associations with disease severity and long-term disease progression. The predictive accuracy of these prognostic models, however, is expected to be lower

compared to the reported sensitivity and specificity of the diagnostic models, as individual variability will be higher and extend across a relatively broad range.

Intestinal organoids as predictive biomarker for CFTR modulators

In the context of short-term CFTR modulator treatment response, several studies showed that average FIS response to CFTR modulators was correlated with short-term clinical drug response across groups with different genotypes [35,49] and in individuals with a variety of CFTR mutations [36]. The FIS assay demonstrated a high predictive accuracy in this context, with a sensitivity of 70-80% and specificity of 100% when selecting the most optimal cut-off value of the FIS assay to define treatment responders [36]. On the other hand, other exploratory studies did not detect an association of FIS with short-term clinical response to LUM/IVA in pwCF homozygous for F508del [30], heterozygous for the A455E mutation [34] or to IVA in people with residual CFTR-function mutations [50]. **Chapter 5** also did not demonstrate an association of FIS response to LUM/IVA and the change in long-term FEV1 decline after LUM/IVA initiation, which was not statistically significant on a group-level.

So how can these observations regarding the strong individual-level associations of FIS in the context of disease severity and progression be explained, with respect to the contradicting results for CFTR modulator treatment response within treatment populations carrying identical genotypes? First, the ability of FIS to detect individual-level associations depends on the total bandwidth of FIS- and clinical responses across the study population, which is considerably larger in populations with varying genotypes, clinical phenotypes and different effect sizes of tested CFTR modulators [**chapter 3**];[36], compared to populations with identical genotype classes and relatively limited variation in disease severity, in which the effect of only one drug is being tested [**chapter 5**];[30,34,50]. Furthermore, the relative contribution of CFTR-dependent factors that can be quantified with FIS to individual variability in short- and long-term clinical response measures is probably limited compared to the impact of other non-CFTR dependent factors. Currently, additional studies are being conducted to assess the predictive capacity of FIS in pwCF carrying at least one F508del mutation who are treated with ETI, which may provide further insights in these hypotheses.

Performance of intestinal organoid assays compared to other human bioassays of CFTR function

The sweat test is the most well-known and widely used *in vivo* biomarker of CFTR function, which quantifies the chloride concentration in sweat (SwCl). The sweat test mainly has a proven track record in the context of CF diagnosis and as such, it is

implemented in global diagnostic guidelines [51,52]. Although SwCl discriminates between different phenotypes on a group-level (e.g. pancreas insufficient or sufficient CF, CFTR-related disorder (CFTR-RD), CF carriers and healthy controls) [43], individual-level relationships have been difficult to establish due to high intrinsic variability across and within these groups [53]. Subsequently, CF diagnosis remains particularly challenging in individuals carrying one or two rare CFTR mutations and an inconclusive sweat test (SwCl 30-60 mmol/L). In the context of prognosis, some studies have suggested that SwCl could stratify groups of pwCF by some clinical measures of disease severity [47,54], yet the association of SwCl with severity of lung disease and other manifestations of long-term disease progression were absent on both a group-level and individual-level [chapter 3];[54]. Similarly, the sweat test demonstrated responsiveness to different CFTR modulators at a population-level [11–15,20], but SwCl only showed a weak correlation with pulmonary outcomes on an individual level in post-hoc analyses of pooled IVA trials in different sub-populations [55].

Intestinal current measurements (ICM) and nasal potential difference (NPD) are CFTR function biomarkers which also have been validated in the context of CF diagnosis. ICM and NPD respectively measure the *ex vivo* electrical current or *in vivo* voltage potential resulting from epithelial ion fluxes at the mucosal surface [43]. In general, both tests are only applied in specialized centers when genetics and sweat tests are inconclusive [51,52]. These biomarkers can accurately characterize individuals at the extreme ranges of CFTR function (e.g. healthy vs. pancreatic insufficient CF), but are less reliable when rare CFTR mutations are present, particularly when clinical features are atypical. Although some studies have also suggested a link of ICM with CF disease severity [47,56], no studies have been performed to examine the association with long-term disease progression. Both biomarkers have shown responsivity to CFTR modulating drugs, but individual correlations to clinical outcomes such as change in FEV1 have not been observed [24,28,30,57,58].

Together, these results indicate that group-level associations with clinical outcomes and responses to CFTR modulators have been established for SwCl, ICM and NPD, but no convincing associations on an individual level. Few studies have evaluated the association of the intestinal organoid assays with these three CFTR function biomarkers or directly compared the performance of these assays. Residual CFTR function measurements by FIS were moderately correlated with SwCl [35,47,49] and ICM [35,47], but FIS responses to dual CFTR modulators were not associated with changes in SwCl, ICM and NPD [30]. Considering predictive performance, the study described in **chapter 3** has been the first to directly compare the performance

of FIS with SwCl on long-term multi-organ disease progression, which clearly favored FIS over SwCl.

As the FIS assay is completely CFTR-dependent with a limited biological variability [35,46], reliability of FIS as a CFTR function biomarker may be stronger than the reliability of other CFTR function biomarkers, which are all subject to a relatively high intrinsic variability due to the influence of non-CFTR dependent factors [43,47,53,59,60]. In terms of feasibility, the sweat test is a relatively easy, cheap and non-invasive procedure which is widely standardized and implemented as a result of its long history in CF diagnosis. ICM, NPD and FIS are all advanced procedures requiring extensive training and are therefore only performed in specialized CF centers. Yet a major advantage of the FIS assay is that intestinal organoids can be cultured and stored in a biobank for future use, allowing for repeated and high-throughput *in vitro* drug testing after a single biopsy procedure, whereas ICM requires a fresh biopsy for every test and NPD can only measure *in vivo* drug effects. On the other hand, results of ICM and NPD are directly available, whereas the culturing and testing procedure in intestinal organoids can take 6-8 weeks.

Qualification of the intestinal organoid model as biomarker of CFTR function and its potential as surrogate endpoint

Biomarkers of CFTR function have the potential to support and accelerate market approval of new targeted therapies in CF when they are qualified as surrogate endpoint. The FDA-NIH biomarker working group defines a validated surrogate endpoint as an endpoint supported by a clear mechanistic rationale and clinical data providing strong evidence that an effect on the surrogate endpoint predicts a specific clinical benefit [61]. As a validated surrogate endpoint, a biomarker can support market approval without the need for additional studies to demonstrate clinical benefit. Without sufficient evidence of a clinical benefit, the biomarker may be qualified as 'reasonably likely surrogate endpoint' or 'candidate surrogate endpoint'. In this case, the biomarker can still support accelerated drug approval, but post-marketing confirmatory trials are required to verify the anticipated effect on clinical benefit [61].

So what do the results regarding the validity and sensitivity of intestinal organoids as CFTR function biomarker suggest? And what additional work would be needed to move forward to the application as a surrogate outcome in clinical trials? For the

latter purpose, the predictive and, to a lesser extent, the prognostic capacity of FIS will be the most important focus.

Recently, the EMA has provided a positive advice concerning the FIS assay as candidate biomarker of CFFTR function, supporting further validation [62]. FIS was deemed a reproducible *ex vivo* biomarker with strong biological rationale, yet direct qualification of the FIS assay as prognostic and predictive biomarker was considered premature based on currently available data. Due to the relatively large contribution of non-CFTR dependent factors to accepted clinical endpoints such as FEV1, accurate predictions of individual outcomes may remain difficult solely based on CFTR function biomarkers. Furthermore, establishing unambiguous definitions of a clinical response and *in vitro* drug response will stay a challenging endeavor.

To summarize, more evidence needs to be generated to obtain official qualification of FIS as a validated surrogate endpoint, at least according to current traditional rules and regulations. This will require additional studies in large and diverse populations, including pwCF with a spectrum of CFTR mutations and different types of CFTR modulators. These studies should further crystallize the capacity of FIS to predict clinical efficacy of CFTR modulating drugs in individuals across and within groups with different disease characteristics on multi organ endpoints. An upcoming European phase 2b multi-center placebo-controlled cross-over trial (CHOICES), in which a novel triple CFTR modulator will be studied in pwCF carrying rare CFTR mutations who are selected based on FIS response, will hopefully provide the first additional insights in the predictive value of FIS.

Given the changing characteristics of the current CF population, however, the question arises whether the required large-scale validation will be feasible according to current standards. Since pulmonary symptoms and function have drastically improved in pwCF using ETI, the focus of future trials will shift from traditional pulmonary clinical outcomes to different outcome measures that will be more sensitive and relevant, at least within this new population. Therefore, it may not be feasible to evaluate individual level associations with pulmonary outcomes in future trials that will be conducted in pwCF who are already using ETI. Additionally, the key role of pulmonary outcome measures as reference standard of clinical response may eventually need to be revised, as they may not be the most important and relevant measures of disease severity and treatment response anymore in the highly effective CFTR modulator era.

III. PERSONALIZATION OF PATIENT-REPORTED OUTCOME MEASURES

Patient-reported outcome measures (PROMs) have been developed to capture relevant health benefits from a patient's perspective and play an increasing role in medical research and care. Due to the expected inhibition of disease progression and improved survival in pwCF using highly effective CFTR modulator therapy, the focus of future research and care will further shift towards improvement of quality of life. In addition to regular clinical endpoints, traditional generic and disease-specific PROMs also might lose their sensitivity and relevance in the current CF population.

This section will discuss the benefits of a personalized approach in the assessment of quality of life and the potential applications of a personalized PROM in future trials and healthcare.

Added value of a personalized PROM in CF

Historically, the Cystic Fibrosis Questionnaire Revised (CFQ-R) has been the first disease-specific PROM developed and validated to assess health-related quality of life in CF [63,64]. Nowadays, it is still the most frequently used PROM in CF research and care. The CFQ-R respiratory domain score is usually selected as secondary endpoint in clinical trials due to its sensitivity and relationship with respiratory symptoms. Yet the majority of pwCF do not consider respiratory symptoms as important and relevant to their quality of life, as emphasized by the results of **chapter 6**, showing that only 9% of study participants chose to prioritize personal quality of life factors related to lung problems. Furthermore, the CFQ-R in general, including the other subdomain scores, is deemed irrelevant by pwCF [65], as the therapeutic advances over the last decades have already changed the lives of pwCF markedly compared to the time period when the CFQ-R was developed and validated.

Chapter 6 showed that a personalized electronic PROM such as the Q-Life app is a reliable, valid and sensitive tool to capture what really matters to individuals with CF in terms of their personal quality of life. The Q-Life app therefore provides the opportunity to measure all relevant and important aspects extending beyond the pulmonary domain. Sensitivity of personal Q-Life scores was comparable to the CFQ-R respiratory domain score in the context of highly effective CFTR modulator treatment (ETI), but remarkably higher than reported for other non-respiratory CFQ-R domain scores in the ETI trial [66];[**chapter 6**]. A personalized PROM could therefore be more relevant and sensitive than traditional PROMs. Furthermore, it

may better accommodate to the heterogeneous and rapidly changing CF population, making such a flexible tool more future-proof than developing novel standardized disease-specific questionnaires, which may quickly become outdated again.

Application in clinical trials and healthcare

Clinical trials

PROMs should be thoroughly validated and approved by regulatory authorities to be applied as endpoints in clinical trials and to support pharmaceutical labeling claims [67]. The study described in **chapter 6** marks the first step in this validation process, but additional large-scale studies will be required in different populations and settings to assess external validation.

The FDA acknowledges that different types of PROMs ask for different validation approaches [67]. For the Q-Life app, the development process and the definition of content validity deviate from common quality of life questionnaires, as pwCF can decide for themselves what is important for their quality of life and what is relevant to measure. Although the Q-Life app showed an excellent sensitivity for highly effective CFTR modulator therapy, further studies should also assess the sensitivity for other therapies that may be less effective, and a minimal clinical important difference should be established [68].

The reported ceiling effects [**chapter 6**] may increase in a population with milder disease symptoms, but this would require further research. The ceiling effects could be decreased by changing to a different scale (e.g. a numeric 0-10 scale instead of a 5-point Likert scale). Other options would be to change the instructional method, asking participants to set specific quality of life-related goals instead of just defining what is most important to their quality of life, or to select the most responsive categories. These strategies may increase the sensitivity of the Q-Life app, but that would be at the expense of relevance.

Healthcare

In a clinical setting, the Q-Life app can be a valuable tool to enhance shared decision-making, as it could be used to guide the conversation between patients and caregivers, creating the opportunity to align care with the patient's own priorities [69]. In addition, the Q-Life app may not only be used to capture treatment benefit, but could also assess the impact of other aspects such as life events on quality of life. As personal priorities are expected to change throughout life, long-term use in a care setting will be facilitated by allowing users to adjust their personal quality of life factors. Therefore, this will be a next step in further development. Moreover, the option to involve other people who are closely related to the patient in a quality

of life assessment will be explored, which may be particularly relevant for children. In general, an app is suitable for clinical and remote monitoring, but integration in electronical data capturing systems and medical records would ultimately facilitate implementation of the Q-Life app in clinical trials and healthcare. Interestingly, the devices on whjch apps are running also offer further opportunities to collect complimentary data from device settings or additional monitoring apps (e.g. activity indicators, heart rate). Because of its individualized properties, future research should elucidate whether a patient-specific PROM like the Q-Life app may also be of added value for other chronic diseases. Similar to generic PROMs, this personalized approach could ultimately allow for comparison of outcomes across different diseases and medical disciplines, but with greater sensitivity and relevance.

IV. CONCLUSION AND RECOMMENDATIONS

CF research and care have entered a new era since the emergence of highly effective CFTR modulator therapy. Traditional pulmonary endpoints are becoming even less sensitive and less relevant in the changing CF population, which consists of individuals with CF who are not eligible for tolerant of CFTR modulators, eligible people without access and those requiring additional therapies due to limited responsiveness to CFTR modulators. To find the most effective drug or combination of drugs for each individual with CF, we will need to leave traditional evidence-based medicine and adopt alternative endpoints and unconventional strategies that can facilitate precision medicine at the level of the individual. Long-term real-world data are essential to predict the actual impact of new treatments on individuals with CF, and act as reference standard to which short-term endpoints such as CFTR function biomarkers and PROMs can be validated as to support individual clinical-decision making.

This thesis showed how short-and long-term real-world effectiveness of dual CFTR modulators compares to efficacy in clinical trials, and provided insights in the challenges of predicting individual treatment responses. Ultimately, long-term real-world data is expected to be very useful to innovate clinical trial design and to identify alternative endpoints that are relevant and sensitive in the target population. To accomplish this, the utilization of different real-world data sources need to be maximized and combined with all available data extending beyond clinical, biological and patient-reported data. This will require supportive technology and analysis methods that are able to connect and handle high-dimensional data. Furthermore, data quality should be warranted to produce reliable predictions.

In addition to its broad application in pre-clinical drug development, the intestinal organoid model could play an important role in the clinical drug development process, but requires more extensive validation to serve as surrogate endpoint in clinical trials. This thesis demonstrated that intestinal organoid swelling is associated with long-term CF disease progression in multiple organ systems, emphasizing its potential as prognostic CFTR function biomarker. The role of intestinal organoid model as predictive biomarker and its context-of-use should be further elucidated in variable, representative and large study populations.

Finally, this thesis showed how close collaboration between patients, healthcare providers, researchers and IT-developers resulted in a novel validated personalized electronic PROM that is able to capture what really matters for individuals with CF. Such personalized tools are able to accommodate to the heterogeneous CF population and have the potential to drive the shift towards more patient-centered research and healthcare.

In this new era of highly effective CFTR modulators, a tailored approach is needed for optimal care of all individuals with CF. For an increasingly large proportion of patients receiving highly effective disease-modifying treatments, disease management may simply shift from intensive hospital care to low care conditions. Yet, for people devoid of disease-modifying treatments or who receive treatments with limited or organ-specific effectiveness, a selection of optimal treatment will be required to maximize individual benefit. As suggested in this thesis, extensive datasets studying various long-term outcomes in real-life settings are likely needed to identify at what precision individual disease states and therapeutic responses can be resolved and predicted through a combination of CFTR and non-CFTR dependent individual assessments.

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CHAPTER 8

Dutch summary / Nederlandse samenvatting

HOOFDSTUK 1. INTRODUCTIE

Voor de behandeling van zeldzame erfelijke ziekten zoals taaislijmziekte (Cystic Fibrosis, afgekort CF) is vaak een individuele aanpak nodig, omdat de ziekte zich bij elke persoon anders kan uiten en omdat iedereen verschillend reageert op medicijnen. Het ontwikkelen van een effectieve behandeling op maat is echter een grote uitdaging, omdat uitkomsten van traditionele kortetermijnstudies zich vaak lastig laten vertalen naar langetermijnuitkomsten van een individu in de dagelijkse praktijk.

In het afgelopen decennium zijn er voor mensen met CF nieuwe medicijnen op de markt gekomen, die gericht zijn op het herstellen van de werking van het *cystic fibrosis transmembrane conductance regulator* (CFTR)-eiwit. Deze medicijnen, genaamd CFTR-modulatoren, hebben aanzienlijke verbeteringen teweeggebracht in het leven van de mensen met CF die deze behandeling krijgen.

Toch is het nog steeds hard nodig om nieuwe behandelingen te blijven ontwikkelen. Ten eerste, omdat ca. 20-30% van de mensen met CF niet in aanmerking komt voor de huidige CFTR-modulatoren en dus nog helemaal geen gerichte behandeling kan krijgen. Ten tweede, omdat deze medicijnen erg duur zijn en wereldwijd nog lang niet overal beschikbaar zijn gekomen of vergoed worden voor de ~70-80% van de mensen met CF die er eigenlijk wel voor in aanmerking komt. En ten derde, omdat het effect van deze modulatoren sterk verschilt tussen individuen.

Om voor deze uiteenlopende groep mensen met CF de beste individuele behandeling te ontwikkelen en te selecteren, is het van belang om het ziektebeloop en de reactie op behandeling op de lange termijn in de dagelijkse praktijk te kunnen voorspellen.

Dit proefschrift beschrijft eerst wat de aandoening CF inhoudt en geeft een overzicht van de huidige beschikbare en potentiële nieuwe medicijnen (**hoofdstuk 2**). Vervolgens wordt onderzocht of intestinale organoïden (zogenaamde mini-darmpjes) en andere klinische tests het ziektebeloop en de reactie op CFTR-modulatoren op de lange termijn kunnen voorspellen (**hoofdstuk 3 t/m 5**). Daarna wordt een nieuwe methode beschreven om op een gepersonaliseerde manier kwaliteit van leven te meten (**hoofdstuk 6**). Tot slot worden de belangrijkste conclusies en aanbevelingen voortkomend uit dit proefschrift besproken (**hoofdstuk 7**).

HOOFDSTUK 2. EEN NIEUW TIJDPERK VOOR MENSEN MET CF

CF is een zeldzame, autosomaal recessieve erfelijke aandoening veroorzaakt door een mutatie in het CFTR-gen. Dit betekent dat de ziekte CF tot uiting komt wanneer iemand een afwijkend CFTR-gen erft van beide ouders. Afhankelijk van de afwijking in het CFTR-gen wordt er geen, weinig, of niet goed werkend CFTR-eiwit geproduceerd. Hierdoor raakt het water- en zouttransport in de lichaamscellen verstoord, waardoor taai slijm ophoopt en verschillende organen aantast. De meest ernstige en levensbedreigende symptomen kunnen ontstaan door aantasting van de longen, lever, alvleesklier en darmen. Daarnaast kunnen er andere problemen optreden zoals bijvoorbeeld botontkalking. Ook is er vaak sprake van onvruchtbaarheid bij mannen of verminderde vruchtbaarheid bij vrouwen. Er bestaan meer dan 2000 verschillende CFTR-mutaties, waarbij de ziekte CF zich op veel verschillende manieren kan uiten en de ernst van de ziekte sterk verschilt per persoon.

Wereldwijd zijn er ongeveer 100.000 mensen met CF, maar dit aantal zal de komende jaren waarschijnlijk toenemen dankzij een stijgende levensverwachting ten gevolge van de verschillende CFTR-modulatoren die in de afgelopen 10-15 jaar zijn ontwikkeld. Waar voorheen alleen behandelingen bestonden om symptomen te bestrijden, zijn deze CFTR-modulatoren gericht op het verbeteren van de werking van het CFTR-eiwit. Hierdoor pakken deze medicijnen doelgericht het onderliggende probleem van de ziekte aan.

De eerste generatie CFTR-modulatoren was de eerste groep doelgerichte medicijnen die beschikbaar kwam voor mensen met de meest voorkomende CFTR-mutatie (de F508del-mutatie). De CFTR-modulatoren van de eerste generatie bestaan uit een combinatie van twee verschillende middelen die de vorm en functie van het CFTR-eiwit samen verbeteren, genaamd lumacaftor/ivacaftor (LUM/IVA) en tezacaftor/ivacaftor (TEZ/IVA). De effectiviteit van deze middelen is echter beperkt, met een verbetering in de longfunctie van ca. 3-4% op de korte termijn. Het laatste nieuwe middel wat recent op de markt is gekomen, genaamd elexacaftor/tezacaftor/ivacaftor, is met een verbetering in longfunctie van ca. 14% veel effectiever en wordt beschouwd als een CFTR-modulator van de tweede generatie. Voor alle modulatoren varieert de mate van verbetering echter sterk per persoon en is het moeilijk te voorspellen wie goed reageert of niet. Kennis over de langetermijneffecten in de praktijk is daarnaast beperkt, omdat de middelen nog relatief kort op de markt zijn en elkaar snel hebben opgevolgd.

Momenteel zijn er nog geen effectieve doelgerichte medicijnen voor ca. 20-30% van de mensen met CF. Zij hebben zeldzamere mutaties in het CFTR-gen, waardoor CFTR-modulatoren bij hen niet werken, of waarvan we nog niet weten of ze hiervoor werken. Voor deze groep mensen worden verschillende soorten nieuwe medicijnen ontwikkeld en getest in klinische studies, maar deze zijn voorlopig nog niet op de markt en waarschijnlijk ook nog niet voor iedereen even effectief.

HOOFDSTUK 3. UITDAGING I: ZIEKTE-UITINGEN VAN CF OP DE LANGE TERMIJN VOORSPELLEN

Voor CF zijn er verschillende tests ontwikkeld die de functie van het CFTR-eiwit kunnen meten, zogenaamde CFTR-functiebiomarkers. Door recente technologische ontwikkelingen kunnen we tegenwoordig stamcellen afnemen van een persoon en hiervan mini-organen, of zogenaamde organoïden, kweken in het laboratorium. De afgelopen jaren is er een test ontwikkeld waarmee de CFTR-functie in darmorganoïden gemeten kan worden. Deze test is gebaseerd op de mate van zwelling van deze darmorganoïden na stimulatie met het stofje forskoline (de FIS-test). Dit stofje zorgt voor activatie van het CFTR-eiwit, waardoor water en zout de organoïden in stroomt zodat deze opzwellen, afhankelijk van hoe goed dit eiwit functioneert. Verschillende kleine kortetermijnstudies hebben eerder al laten zien dat deze CFTR-functiemetingen in darmorganoïden van een individu met CF geassocieerd zijn met de ernst van de ziekte, zowel bij kinderen met verschillende CFTR-mutaties als bij volwassenen met twee F508del-mutaties.

In dit hoofdstuk onderzochten we voor het eerst de relatie van de FIS-test in darmorganoïden met ziekte-ernst en achteruitgang van orgaanfunctie op de lange termijn in een grote groep mensen met CF van verschillende leeftijden en met verschillende CFTR-mutaties. We vonden dat de FIS-test geassocieerd was met achteruitgang van longfunctie, een verminderde werking van de alvleesklier en het ontwikkelen van leverziekte, onafhankelijk van bijvoorbeeld de leeftijd en het type CFTR-mutatie. De zweettest, ook een CFTR-functietest die wereldwijd als gouden standaard gebruikt wordt voor het stellen van de diagnose CF, was niet geassocieerd met het ziektebeloop op de lange termijn. Deze resultaten suggereren dat de FIS-test in darmorganoïden een betere voorspeller is van het langetermijnziektebeloop van CF dan de zweettest. Dit betekent dat we de FIS-test ook daadwerkelijk in de praktijk kunnen gebruiken ter ondersteuning van de diagnose CF en de patiënt beter kunnen informeren over het te verwachten ziektebeloop, ofwel de prognose, op de lange termijn.

HOOFDSTUK 4. LANGETERMIJNEFFECTEN VAN DE EERSTE GENERATIE CFTR-MODULATOREN

LUM/IVA en TEZ/IVA zijn doelgerichte medicijnen van de eerste generatie CFTR-modulatoren die als eerst op de markt kwamen voor mensen met CF met twee F508del-mutaties. In dit hoofdstuk analyseerden we de langetermijnveranderingen in verschillende klinische uitkomsten tot 3 jaar na start van deze CFTR-modulatoren in vergelijking met de jaren daarvoor. Hiervoor maakten we gebruik van klinische data uit de dagelijkse praktijk van alle mensen met CF in Nederland, die jaarlijks systematisch verzameld worden in de Nederlandse CF-Registratie door de Nederlandse CF Stichting.

We vonden dat longfunctieachteruitgang op de lange termijn minder werd in de jaren na start van CFTR-modulatoren, ten opzichte van de jaren ervoor. Bij volwassenen veranderde de trend in BMI niet, maar de BMI Z-score, die bij kinderen gemiddeld jaarlijks licht daalde voor start van behandeling, liet wel een duidelijke verbetering zien in de jaren daarna. In het eerste jaar na start van CFTR-modulatoren verminderde daarnaast het gemiddelde aantal dagen dat mensen met CF behandeld werden met antibiotica via het infuus. Zo'n behandeling wordt meestal gegeven vanwege een pulmonale exacerbatie, ofwel een plotselinge toename van longklachten die vaak samenhangt met achteruitgang van de longfunctie. Echter in de jaren daaropvolgend nam het gemiddelde aantal dagen antibiotica via het infuus weer geleidelijk toe, op hetzelfde tempo als in de jaren voor start van de behandeling. De verandering in longfunctie en antibiotica via het infuus varieerde ook sterk tussen groepen mensen met CF en verschillende longfuncties.

Als we deze resultaten vergelijken met de resultaten van de oorspronkelijke geneesmiddelenstudies die zijn uitgevoerd in specifieke omstandigheden met een uitgeselecteerde groep mensen met CF, dan lijkt de verbetering van verschillende klinische uitkomsten na start van de eerste generatie CFTR-modulatoren in de dagelijkse praktijk minder sterk te zijn. Deze uitkomsten variëren ook flink tussen mensen met CF en verschillende eigenschappen. Data uit de dagelijkse praktijk zijn dus belangrijk om een betere inschatting te kunnen maken van het daadwerkelijke effect dat we kunnen verwachten van een doelgericht medicijn op een individu.

HOOFDSTUK 5. UITDAGING II: LANGETERMIJNEFFECTEN VAN DE EERSTE GENERATIE CFTR-MODULATOREN VOORSPELLEN

Vervolgens bestudeerden we mogelijke factoren die de effecten van de eerste generatie CFTR-modulatoren op de lange termijn zouden kunnen voorspellen bij mensen met CF en de dubbele F508del-mutatie. We keken hierbij vooral naar de voorspellende waarde van de FIS-test in darmorganoïden, maar ook naar andere klinische meetwaarden, zoals leeftijd, geslacht, de zweettest en het aantal pulmonale exacerbaties per jaar dat behandeld moet worden met antibiotica via het infuus.

Dit onderzoek vond echter geen duidelijke verbetering van de achteruitgang in longfunctie in de 3 jaar na start met de eerste generatie CFTR-modulatoren ten opzichte van de 3 jaar daarvoor. De FIS-test was in dit geval niet voorspellend voor de mate van verandering in het longfunctieverlies op de lange termijn, het aantal pulmonale exacerbaties behandeld met antibiotica via het infuus of voor de mate van verandering in de zweettest op de korte termijn. In de dagelijkse praktijk valt de langetermijnverbetering in longfunctie na start van de eerste generatie CFTR-modulatoren bij mensen met de dubbele F508del-mutatie dus tegen en blijf het moeilijk te voorspellen voor het individu. Vervolgonderzoek is nodig om erachter te komen hoe dat zit voor mensen met andere CFTR-mutaties en voor middelen die effectiever zijn, zoals de tweede generatie CFTR-modulatoren.

HOOFDSTUK 6. UITDAGING III: METEN WAT ER VOOR DE PATIËNT TOE DOET – EEN GEPERSONALISEERDE APP OM KWALITEIT VAN LEVEN TE METEN

Naast biologische metingen zoals de FIS-test en klinische metingen zoals longfunctie, is het belangrijk om te meten welk effect een behandeling heeft op het leven en welzijn van de patiënt. Hiervoor wordt doorgaans gebruik gemaakt van gevalideerde vragenlijsten gericht op kwaliteit van leven. Bijna 25 jaar geleden is voor mensen met CF de *Cystic Fibrosis Questionnaire* (CFQ) ontwikkeld, met het doel de kwaliteit van leven van mensen met CF systematisch te meten op verschillende domeinen. Deze vragenlijst wordt nog steeds gezien als de gouden standaard en als zodanig veelvuldig gebruikt in de meeste geneesmiddelenstudies voor CF. De focus ligt hierbij doorgaans op het respiratoire domein van de CFQ, waarin gevraagd wordt naar longgerelateerde symptomen zoals hoesten en benauwdheid. De vragen in de CFQ zijn echter gedateerd en voor veel mensen met CF niet (meer) relevant of

belangrijk voor hun kwaliteit van leven.

In dit onderzoek ontwikkelden we in samenwerking met mensen met CF de Q-Life app. Dit is een gepersonaliseerde app waarin mensen met CF zelf 3-5 factoren kunnen beschrijven die zij belangrijk vinden voor hun kwaliteit van leven en kunnen bijhouden in welke mate ze hierin door CF worden beperkt. Hiermee ligt de focus dus alleen op de dingen die er voor het betreffende individu toe doen. Vervolgens werd de Q-Life app in twee verschillende studies gebruikt door mensen met CF en werden de testeigenschappen vergeleken met die van de CFQ. We vonden dat de Q-Life app een betrouwbare, valide en gevoelige meetmethode is om kwaliteit van leven op een gepersonaliseerde manier te meten. De Q-Life app zou dus een veelbelovend uitkomstmaat kunnen zijn waarmee we de behandeling op maat kunnen verbeteren.

HOOFDSTUK 7. CONCLUSIE

Om ieder individu met CF de meest optimale behandeling te kunnen geven, is het belangrijk om het effect van medicijnen goed te kunnen meten en te voorspellen. Hiervoor zijn gevoelige tests nodig die geassocieerd zijn met relevante uitkomsten op de lange termijn.

Dit proefschrift laat zien dat de FIS-test in darmorganoïden een veelbelovende test lijkt te zijn om de mate van ziekte-ernst en achteruitgang op de lange termijn te voorspellen. Het voorspellen van de individuele langetermijnrespons op CFTR-modulerende behandelingen op blijft vooralsnog echter een uitdaging met de huidige tests en uitkomstmaten die momenteel voorhanden zijn. Nieuwe gepersonaliseerde patiënt-gerapporteerde uitkomstmaten zoals de Q-Life app kunnen een belangrijke toevoeging zijn om het effect van nieuwe medicijnen op het leven van een individu beter te meten.

Het voorspellen van de toekomst zal altijd gepaard gaan met een bepaalde mate van onzekerheid. Vervolgonderzoeken waarbij gebruik gemaakt wordt van innovatieve analysemethoden, grote datasets met langetermijngegevens vanuit verschillende databronnen en alternatieve uitkomstmaten gericht op een combinatie van CFTR- en niet-CFTR-afhankelijke persoonsgebonden factoren, zullen moeten uitwijzen met welke mate van zekerheid ziektebeloop en respons op behandeling te voorspellen zijn voor een individu met CF.

APPENDICES

Abbreviations
Contributing authors
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ABBREVIATIONS

AUC	Area under the curve
BMI	Body mass index
cAR1	First-order auto-regressive correlation structure
CBAVD	Congenital bilateral absence of the vas deferens
CF	Cystic fibrosis
CFQ-R	Cystic fibrosis questionnaire-revised
CFRD	Cystic fibrosis-related diabetes
CFRLD	Cystic fibrosis-related liver disease
CFSPID	Cystic fibrosis screen positive, inconclusive diagnosis
CFTR	Cystic fibrosis transmembrane conductance regulator
CFTR-RD	Cystic fibrosis transmembrane conductance regulator-related disorder
CI	Confidence interval
CRMS	CFTR-related metabolic syndrome
DIOS	Distal intestinal obstruction syndrome
EMA	European medicines agency
ePROM	Electronic patient-reported outcome measure
ETI	Elexacaftor/tezacaftor/ivacaftor
FDA	Food and drug administration
FEV_1	Forced expiratory volume in 1 second
FEV_1% pred	Forced expiratory volume in 1 second, percentage of predicted
FEV_1pp	Forced expiratory volume in 1 second, percentage of predicted
FIS	Forskolin-induced swelling
GLI	Global lung function initiative
ICC	Intraclass correlation coefficient
ICM	Intestinal current measurement
IQR	Interquartile range
IRB	Institutional review board
IRR	Incidence rate ratio
IRT	Immunoreactive trypsinogen
IV	Intravenous
IVA	Ivacaftor
LUM	Lumacaftor
NBS	Newborn screening
NPD	Nasal potential difference
OR	Odds ratio

PEx	Pulmonary exacerbation(s)
PI	Pancreas insufficient
$ppFEV_1$	Forced expiratory volume in 1 second, percentage of predicted
PROMs	Patient-reported outcome measures
PS	Pancreas sufficient
PTC	Premature termination codon
pwCF	People with cystic fibrosis
RCT	Randomized controlled trial
rhDNase	Recombinant human deoxyribonuclease
SCC	Sweat chloride concentration
SD	Standard deviation
SLA	Steady-state lumen area
SwCl	Sweat chloride concentration
TEZ	Tezacaftor
VX-445	Elexacaftor
VX-661	Tezacaftor
VX-770	Ivacaftor
VX-809	Lumacaftor
WHO	World health organization

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ACKNOWLEDGEMENTS / DANKWOORD

Het einde van een tijdperk: het proefschrift is af, mijn promotietraject zit er nu echt op. Mede dankzij de hulp en steun van vele anderen was het een onvergetelijk mooie en leerzame periode. Precies zoals ik vooraf gehoopt had. Ik wil iedereen die hieraan heeft bijgedragen enorm bedanken, met een aantal mensen in het bijzonder.

Alle mensen met CF en ouders van kinderen met CF die hebben deelgenomen aan de onderzoeken of hebben geparticipeerd als vertegenwoordiger: bedankt voor jullie bijdrage en tomeloze inzet om de zorg voor mensen met CF te blijven verbeteren en toe te werken naar een effectief medicijn voor iedereen.

Kors: het was af en toe even zoeken naar de juiste pagina in onze gebruiksaanwijzingen, maar mede dankzij jouw geduld en streven naar de juiste behandeling op maat (zowel voor je patiënten als voor je promovendi) werd onze samenwerking een succes. Ik ben ontzettend dankbaar voor de vrijheid die je me vanaf dag 1 hebt gegeven om mijn eigen weg te zoeken en zo nodig de gebaande paden te verlaten. Ook al heb ik je haar steeds grijzer zien worden in mijn jaren als PhD-student, je bleef me altijd aanmoedigen om eigenwijs te zijn en gaf niet alleen de ruimte om anders te mogen denken, maar waardeerde dat ook. Ik bewonder je geduld, doorzettingsvermogen en vastberadenheid om alles uit de kast te halen in het belang van de patiënt, waar ik mezelf ook altijd aan herinner als ik af en toe (lees: heel vaak) twijfel of ongeduldig ben.

Jeff: als basale wetenschapper vorm je samen met Kors een mooi promotorenduo. Je bent enorm energiek, enthousiast en draagt je kennis graag over op je promovendi. Ik heb dan ook veel van je geleerd, vooral in de donkere dagen dat we bij revisieronde 3 nog steeds eindeloos aan de ERJ-tabel zaten te sleutelen en alle CFTR-mutaties met bijbehorende classificatie de revue passeerden. Daarnaast heb ik veel bewondering voor je 'subsidieschrijftalent' en ben dan ook enorm dankbaar dat je de tijd en ruimte voor me maakt om hierin van je te kunnen leren. Ondanks onze verschillende persoonlijkheden kunnen we samen altijd goed 'mijmeren over het leven' en weten we elkaar te vinden voor een beetje gezelligheid, waardoor ik me nooit heb hoeven vervelen op de vele congressen, teamuitjes, etentjes en borrels die we hebben gehad.

René: ik ben blij dat onze wegen elkaar hebben gekruist en we de afgelopen jaren hebben samengewerkt aan de complexe analyses in dit proefschrift. Onze wekelijkse digitale afspraken tijdens de COVID-pandemie waren altijd een welkome

onderbreking van de stille en lange thuiswerkdagen, die ik in die tijd voornamelijk doorbracht met mezelf en R. Naast je adviezen bij het programmeren mocht ik graag abstract met je redeneren, wat me vaak verder hielp bij het vormen van mijn ideeën en het bedenken van creatieve oplossingen. Ook als alles op rolletjes liep en ik geen vragen had over mijn codes kletsten we toch altijd even bij, wat ik heel erg heb gewaardeerd. Je was een fantastisch, kundig, bescheiden en behulpzaam mens, ik ben dankbaar je gekend te hebben.

Alle **co-auteurs**: dankjulliewel voor de fijne samenwerking aan alle studies en waardevolle bijdragen aan dit proefschrift.

NCFS: bedankt voor de subsidies die bijgedragen hebben aan de studies in dit proefschrift en de hulp bij het vinden van mensen met CF die konden participeren in het Q-Life project. In het bijzonder heel veel dank aan **Vincent** en **Domenique** voor jullie betrokkenheid bij dit proefschrift. Mede dankzij jullie enthousiasme, behulpzaamheid en het snelle schakelen was het erg prettig om met jullie samen te werken!

Jan Strien, Freek Böhm en alle andere collega's van **Solviteers** die de afgelopen jaren achter de schermen betrokken zijn geweest bij de Q-Life app: als arts-onderzoeker was het een hele ervaring om me te begeven in de wereld van de IT-ontwikkeling. Van sprints tot hamburgermenu's, ik heb veel nieuwe begrippen van jullie geleerd! De ontwikkelfases van de Q-Life app gingen met pieken en dalen, waarbij we regelmatig voor onverwachte 'bugs' kwamen te staan. Dankzij de korte lijntjes en jullie bereidheid om er samen voor de patiënt het beste van te maken konden de meeste problemen vroeg of laat toch worden opgelost en werd het Q-Life project een succes. Bedankt voor de fijne samenwerking.

Gitte, Peter, Hannah, Maaike, Sabine van der Laan en **Marlou**, mijn **collega-promovendi uit het WKZ**! Toen ik in september 2018 in het WKZ begon was de 'KLZ/KA-PhD-kamer' helaas net opgeheven en verhuisden we naar de flexruimte. Al gauw hadden we onze eigen VASTE flexplekken geclaimd en ingericht met foto's, theedozen en andere persoonlijke dingen die ons territorium markeerden. Het was fijn om al het PhD-lief-en-leed met jullie te kunnen delen tijdens de vele koffiepauzes, lunches, borrels en later de digitale koffiedates tijdens de COVID-pandemie. Dankzij jullie positieve mindset en humor ben ik vele kleine en grote PhD-dalen doorgekomen. Ook de onderlinge bereidheid om even te sparren, met elkaar mee te denken en elkaar te helpen heb ik altijd enorm gewaardeerd.

Sabine Michel: vanaf het begin van mijn promotietraject kon ik altijd bij je terecht. Niet alleen met onderzoeksgerelateerde vragen omtrent WMO- en niet-WMO-procedures, METC-indieningen, organisatie en uitvoering van studies, dataverzameling etc. etc., maar ook voor een gezellig kopje koffie of een luisterend oor. Als studiecoördinator binnen de KLZ en het 030-lab ben je van grote waarde en heb je de afgelopen jaren veel bereikt voor de patiënt. Daarnaast heb ik zeker ook genoten van je gezelligheid op de vele congressen en verschillende teamuitjes in binnen- en buitenland.

(Oud-)collega's van het research team: **Hannah** en **Stephan**, we hebben heel wat darmbiopten afgenomen bij mensen met CF. Van jong tot oud, in het Nederlands, Engels, Duits, of met handen en voeten, samen met jullie verliep het altijd op rolletjes. **Emma**, dankjewel voor al het werk dat je achter de schermen verricht bij het controleren van alle studiegegevens.

Collega's van de kinderlongziekten: **Karin, Bert, Sabine Prevaes, Sophie, Tamara, Cora, Marit, Hetty, Joyce** en **Valesca**, bedankt voor het meedenken bij mijn onderzoeken, jullie hulp bij het includeren van patiënten en jullie altijd scherpe en kritische vragen tijdens het refereren op de vrijdagochtend! Ik heb daarnaast ook veel van jullie geleerd tijdens de maanden dat ik bij de kinderlongziekten heb meegedraaid als AIOS. Het heeft mijn klinische blik enorm verruimd en ik kan het iedereen aanraden om jezelf regelmatig buiten de vaste hokjes van je eigen specialisme te begeven.

Myriam: tijdens mijn promotietraject wilde ik nogal eens een afwijkende route kiezen, wat een hele uitdaging kan zijn in het UMC. Zonder jou was ik waarschijnlijk hopeloos verstrikt geraakt in de bureaucratische regels, maar jij wist met jouw engelengeduld en vasthoudendheid vroeg of laat altijd de weg in het doolhof te vinden en kreeg het toch voor elkaar. Daarnaast was je altijd een grote steun en toeverlaat voor iedere PhD, het was een voorrecht om tot aan je pensioen met je te hebben mogen werken!

Wilma: van stagiaire bij het secretariaat kinderlongziekten tot stafsecretaresse, child-healthmedewerkster en longfunctie-ondersteuner, je bent van alle markten thuis! Ook voor mij als PhD-student stond je altijd vliegensvlug klaar om helpen, dingen te regelen of uit te zoeken. Je bent een aanwinst voor het team, dankjewel voor de fijne samenwerking.

Mijn (wetenschaps)studenten: Doris, Joshena, Jasmijn, Tessa en **Barbara**, heel veel dank voor jullie bijdrage aan de onderzoeken die geleid hebben tot dit

proefschrift. Ik heb genoten van jullie verschillende karakters en talenten en heb veel van jullie geleerd. Ik hoop dat ik mijn enthousiasme voor de wetenschap op jullie over heb kunnen dragen.

Collega's van het Beekmanlab: het is mooi om te zien hoe de groep in de afgelopen jaren enorm is gegroeid, met vele **post-doc's, promovendi, analisten** en **studenten**. Ik heb veel van jullie geleerd tijdens de labmeetings op dinsdagochtend en genoten van de gezelligheid op de gezamenlijke congressen, teamuitjes, labiekdagen en PhD-retreats. Ik wil jullie bedanken voor de unieke samenwerking tussen het lab en de kliniek; het moge duidelijk zijn dat dit proefschrift er zonder jullie niet was geweest.

Eyleen: samen hebben we heel wat bloed, zweet en tranen gespendeerd aan ons ERJ-paper. Zelfs tijdens je zwangerschapsverlof heb je nog veel gewerkt aan de revisies van het manuscript. Ik denk dat we elkaar met onze verschillende achtergronden goed hebben aangevuld, waardoor onze samenwerking altijd soepel verliep. Ik ben nog steeds ontzettend trots op het resultaat! Ik ben je daarnaast ook heel erg dankbaar dat je in de COVID-tijd een grote steun was op momenten dat ik niet helemaal lekker in mijn vel zat of even wat extra motivatie of juist afleiding nodig had. Je bent naast een fijne collega ook een fantastisch, lief en warm mens!

Annelotte: toen ik net begon in het WKZ maakte jij mij al gauw wegwijs binnen het Beekmanlab en legde je me de fijne kneepjes van het organoïdenvak uit. Een maand later bleken we als roomies in de Rocky Mountains en Denver ook een match made in heaven, ondanks onze verschillende persoonlijkheden (ik jouw 'oase van rust', jij mijn 'spraakwaterval'). 's Avonds even stoom afblazen over de winterse wandeltochten die we beide als een zware beproeving ervaarden (ik vanwege mijn zomerse outfit, jij omdat alles even tegenzat), gezellig buiten in de jacuzzi bij -10 graden. Toen ik daarna verrassend genoeg ziek werd, kocht je met alle liefde de Wallmart voor me leeg en kwam je aan met allerlei zelfzorgmiddeltjes, in een poging om me weer op te lappen. Mede dankzij jou was het een onvergetelijk eerste congres! In Nashville deden we het nog eens dunnetjes over, maar helaas gooide COVID daarna roet in het eten en is het daar qua congressen bij gebleven. Inmiddels zit je alweer een tijdje helemaal op je plek bij het Julius Centrum en ben ik blij dat we elkaar nog regelmatig spreken, ook al zijn onze dates altijd te kort om helemaal bij te kletsen. Ik zou het super leuk vinden om in de toekomst nog eens samen te werken!

Tessa Nizet en **Henk Codrington**, ik waardeer de ruimte die jullie me hebben gegeven om mijn opleiding tot longarts in het Hagaziekenhuis te onderbreken voor dit promotietraject en voor jullie steun om mijn verdere weg te vinden in het UMC

Utrecht. Heel veel dank voor alles wat ik bij jullie geleerd heb tijdens mijn jaren in het Haga.

Hassan: ook aan jou een woord van dank. Je bent een wandelende encyclopedie, zeer bevlogen dokter die alles over heeft voor zijn patiënten en meestal heerlijk eigenwijs. Je bent een voorbeeld voor vele jonge dokters en hebt mij als mentor ook altijd geïnspireerd. De laatste jaren misschien een beetje uit het oog, maar zeker niet uit het hart. Dankjewel dat ik de laatste periode bij je terecht kon om te sparren over mijn toekomst.

Katrien, Winifred, Stephanie en **Tessa Pletting**: van collega AIOS in het Haga tot vriendinnen, wat fijn om te zien hoe jullie je weg als longarts (bijna) allemaal hebben gevonden en om lief en leed hierover met jullie te kunnen delen. Bedankt voor jullie luisterend oor, gezelligheid en alle aanmoedigingen en support de afgelopen jaren!

Collega's van de longziekten in het UMC Utrecht: wat fijn om met open armen in jullie team te zijn ontvangen en de ruimte te hebben gekregen om mijn promotietraject en de laatste maanden van mijn opleiding bij jullie af te ronden. Bedankt voor alle hulp en steun, jullie zijn een fantastisch team.

Harry: van mijn eerste baan als ANIOS in het Haga tot jouw pensioen als afdelingshoofd in het UMC. Onze wegen hebben elkaar regelmatig gekruist en we delen de drive om het leven van mensen met CF te blijven verbeteren. Ik wil je bedanken voor je hulp bij alle onderzoeken tijdens mijn promotietraject, het was erg gezellig om samen op jouw poli deelnemers te includeren voor het Q-Life project. Daarnaast ook veel dank voor het faciliteren van mijn resterende opleiding in het UMC.

Hans van der Steen en **Johan Pel**: hoewel mijn tijd bij jullie op de afdeling neurowetenschappen in het Erasmus MC al meer dan 10 jaar geleden is, wil ik jullie op deze plaats toch graag bedanken. Mijn liefde voor de wetenschap en de wens om ooit te promoveren zijn namelijk voortgekomen uit de fantastische tijd die ik bij jullie gehad heb tijdens mijn onderzoeksstage binnen de geneeskundestudie – en later als MSc neuroscience student. De gedachten aan het grenzeloze optimisme en enthousiasme van Hans en de rust en wijze lessen van Johan (probeer het glas af en toe ook eens halfvol te zien) hebben mij ook tijdens mijn promotietraject nog regelmatig geholpen mijn motivatie terug te vinden als het even tegenzat, of wanneer ik (iets) te kritisch op mijn eigen resultaten was. Heel veel dank voor de inspiratie en alles wat ik van jullie heb geleerd.

Lieve **Koen** en **Karin**: samen met jullie opgegroeid in het zwembad en alle drie terecht gekomen in de zorg. Het is een voorrecht om al meer dan 20 jaar alles met jullie te kunnen delen. Ik ben dankbaar voor jullie vriendschap en steun en ben blij dat jullie erbij zijn om deze promotiemijlpaal samen te vieren.

Marco G, Suus, Joost, Esther, Mike, Anneke en **Ronnie G**: bedankt voor alle etentjes, feestjes, spelletjesavonden en andere gezelligheid in de afgelopen jaren. Er leek nooit een einde aan te komen, maar zelfs ik ben nu (bijna) klaar met mijn studie!

Lieve **Simone**: in 2006 begonnen we aan geneeskunde in dezelfde studiegroep en al snel waren we onafscheidelijk. We hebben in onze studententijd bijna elke dag met elkaar doorgebracht, inclusief vele weekendjes weg, stedentrips en andere vakanties. Wat was ik blij dat we samen onze wetenschapsstage in het 4e jaar mochten doen en jij daarna ook nog wel een jaartje langer wilde blijven om samen de master neuroscience te volgen. Onze samenwerking in het onderzoek verliep als een hogesnelheidstrein omdat we in veel dingen hetzelfde zijn en handelen, maar elkaar op een paar vlakken ook precies aanvullen. Het was dus op zijn zachtst gezegd flink ontwennen toen we na onze studie allebei een andere weg gingen: jij werd huisarts en ik ging in opleiding tot longarts. Het duurde even voor we onze draai hadden gevonden en onze vriendschap een nieuwe vorm had aangenomen. Intussen hebben we samen veel meegemaakt en ben ik enorm trots op wat je allemaal doet en hebt bereikt. Ik ben heel erg dankbaar voor onze vriendschap en ontzettend blij dat je naast me staat op deze belangrijke dag.

Lieve **Yaggie**: we zaten al een paar jaar bij elkaar op de middelbare school, maar onze vonk sloeg pas over in een stapelbed in Florence, tijdens onze Romereis in de 5e klas. We gingen samen geneeskunde studeren in Rotterdam, ook al kon jij pas een jaar later dan ik beginnen. In al die jaren hebben we veel meegemaakt en ben je er altijd voor me geweest, in goede en slechte tijden, meestal met de slappe lach, soms met een traan van verdriet. We delen (onder andere) de liefde voor de wetenschap en ik heb veel bewondering voor hoe je jouw promotietraject hebt aangepakt en het doorzettingsvermogen en de veerkracht die je daarbij toonde. Je hebt vele talenten die je zelf vaak onderschat. Met veel trots heb ik als paranimf naast je gestaan tijdens jouw verdediging en ik ben dan ook heel blij om jou op deze dag bij me te hebben en deze mijlpaal samen te kunnen vieren.

Mijn schoonfamilie, Rinus, Annelies, Gitte en **Lis**: altijd nieuwsgierig naar mijn belevenissen op het werk en betrokken bij al het wel en wee van mijn promotietraject. **Rinus**, speciale dank voor het uitgebreid reviseren van al mijn

manuscripten op het gebied van English grammar, een waardevolle bijdrage aan dit proefschrift.

Lieve **oma**: misschien wel mijn grootste fan, dankjewel dat je op de achtergrond altijd meeleeft. Ik weet dat je trots op me bent!

Lieve **Anouk** en **Ines**: wat is het fijn om 2 zussen te hebben die precies hetzelfde denken en altijd meteen begrijpen wat je bedoelt, met of zonder woorden. Jullie zijn een enorme steun geweest tijdens dit promotietraject dankzij jullie luisterend oor, scherpe analyses en heldere inzichten over elk probleem of dilemma dat ik ook maar ter tafel bracht. Onze gezamenlijke sportavonden en -ochtenden, waarin we soms zelfs tijdens de trainingen onze gesprekken voortzetten, hebben altijd geholpen om mijn hoofd leeg te maken en hebben mij absoluut door de COVID-tijd heen gesleept. Ik ben trots op de manier waarop jullie altijd jullie eigen weg zoeken, en jullie daarin vooral niet van de wijs laten brengen. Als ik twijfel over mijn pad, denk ik altijd aan jullie.

Lieve **papa** en **mama**, bedankt dat jullie me altijd hebben aangespoord om mijn eigen keuzes te maken en me onvoorwaardelijk hebben gesteund in het bereiken van mijn doelen. Bedankt dat jullie er altijd zijn.

Lieve **Kas**, mijn grote steun en toeverlaat, mijn liefde, mijn thuis. Zonder jou was het nooit gelukt. Ik kijk uit naar de toekomst met ons kleine meisje.

LIST OF PUBLICATIONS

This thesis

Danya Muilwijk, Tessa J. van Paridon, Doris C. van der Heijden, Brenda M. Faber-Bisschop, Domenique D. Zomer-van Ommen, Harry G.M. Heijerman, Cornelis K. van der Ent. Development and validation of a novel personalized electronic patient-reported outcome measure to assess quality of life (Q-LIFE): a prospective observational study in people with Cystic Fibrosis. **eClinicalMedicine** 2023, Jul 27;62:102116. DOI: 10.1016/j.eclinm.2023.102116.

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Danya Muilwijk, Marlou C. Bierlaagh, Peter van Mourik, Jasmijn Kraaijkamp, Renske van der Meer, Rutger M. van den Bor, Harry G.M. Heijerman, Jeffrey M. Beekman, Cornelis K. van der Ent. Prediction of real-world long-term outcomes of people with CF homozygous for the F508del mutation treated with CFTR modulators. **Journal of Personalized Medicine** 2021, Dec 16;11(12):1376. DOI: 10.3390/jpm11121376.

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Other publications

Lidewij W. Rümke, Wouter L. Smit, Ailko Bossink, Gijs J.M. Limonard, Danya Muilwijk, Lenneke E.M. Haas, Chantal Reusken, Sanne van der Wal, Bing J. Thio, Yvonne M.G. van Os, Hendrik Gremmels, Jeffrey M. Beekman, Monique Nijhuis, Annemarie M.J. Wensing, Michiel Heron, Steven F.T. Tijsen. Impaired SARS-CoV-2 specific T-cell response in patients with severe COVID-19. **Frontiers in Immunology** 2023, Apr 17;14:1046639. DOI: 10.3389/fimmu.2023.1046639.

Sacha Spelier, Eyleen de Poel, Georgia N. Ithakisiou, Sylvia W.F. Suen, Marne C. Hagemeijer, Danya Muilwijk, Annelotte M. Vonk, Jesse E. Brunsveld, Evelien Kruisselbrink, Cornelis K. van der Ent, Jeffrey M. Beekman. High-throughput functional assay in cystic fibrosis patient-derived organoids allows drug repurposing. **ERJ Open Research** 2023, Jan 30;9(1):00495-2022. DOI: 10.1183/23120541.00495-2022.

Maarten H. Geurts, Eyleen de Poel, Gimano D. Amatngalim, Rurika Oka, Fleur M. Meijers, Evelien Kruisselbrink, Peter van Mourik, Gitte Berkers, Karin M. de Winter-de Groot, Sabine Michel, Danya Muilwijk, Bente L. Aalbers, Jasper Mullenders, Sylvia F. Boj, Sylvia W.F. Suen, Jesse E. Brunsveld, Hettie M. Janssens, Marcus A. Mall, Simon Y. Graeber, Ruben van Boxtel, Cornelis K. van der Ent, Jeffrey M. Beekman, Hans Clevers. CRISPR-based adenine editors correct nonsense mutations in a Cystic Fibrosis organoid biobank. **Cell Stem Cell** 2020, Apr 2;26(4):503-510e7. DOI: 10.1016/j.stem.2020.01.019.

Danya Muilwijk*, Simone Verheij*, Johan J.M. Pel, Agnita J.W. Boon, Johannes van der Steen. Changes in timing and kinematics of goal-directed eye-hand movements in early-stage Parkinson's disease. **Translational Neurodegeneration** 2013, Jan 9;2(1):1. DOI: 10.1186/2047-9158-2-1. * These authors contributed equally.

Simone Verheij*, Danya Muilwijk*, Johan J.M. Pel, Tischa J.M. van der Cammen, Francesco U.S. Mattace-Raso, Johannes van der Steen. Visuomotor impairment in early-stage Alzheimer's disease: changes in relative timing of eye and hand movements. **Journal of Alzheimer's Disease** 2012, 30(1):131-43. DOI: 10.3233/JAD-20120111883. * These authors contributed equally.

DANYA MUILWIJK

Curriculum Vitae

Personal details

First name	Danya
Surname	Muilwijk
Date of birth	13 August 1988
Place of birth	Dordrecht
Residence	Den Haag
Characteristics	Curious, driven, out of the box, sincere
Interests	Sports, traveling, friends and family

PhD project

PhD pediatric pulmonology – University Medical Center Utrecht **2018 – 2023**
Thesis: Cystic Fibrosis: a real-world challenge to predict individual outcomes
PhD-supervisors: Prof. dr. Kors van der Ent & prof. dr. Jeffrey Beekman
Statistical supervisor: Prof. dr. ir. René Eijkemans
Thesis defense: 27 October 2023

Grants and awards

Award for patient involvement in research **2022**
€15.000,- "Sterk Participatie Prijs" – Dutch Lung Foundation

Cystic Fibrosis Research Grant **2019 & 2020**
€100.000,- "CFOS" – Dutch Cystic Fibrosis Foundation

Clinical training

Pulmonology resident (AIOS) – University Medical Center Utrecht **2023 – 2024**
Expected end date of residency: May 2024

Pulmonology resident (AIOS) – University Medical Center Utrecht **2022 – 2023**
Dept. of pediatric pulmonology

Pulmonology resident (AIOS) – Haga Hospital Den Haag **2017 – 2018**

Pulmonology resident (AIOS) – Reinier de Graaf Gasthuis Delft **2015 – 2017**
Dept. of internal medicine

Pulmonology resident (ANIOS) – Haga Hospital Den Haag **2014 –2015**

Education

MSc in Clinical Epidemiology – Utrecht University **2019 – 2021**
Specialization tracks: clinical epidemiology and medical statistics

MSc in Neuroscience – Erasmus Medical Center Rotterdam **2010 – 2011**

Medicine – Erasmus Medical Center Rotterdam **2006 – 2014**
Cum laude

Gymnasium – Johan de Witt Gymnasium Dordrecht **2000 – 2006**