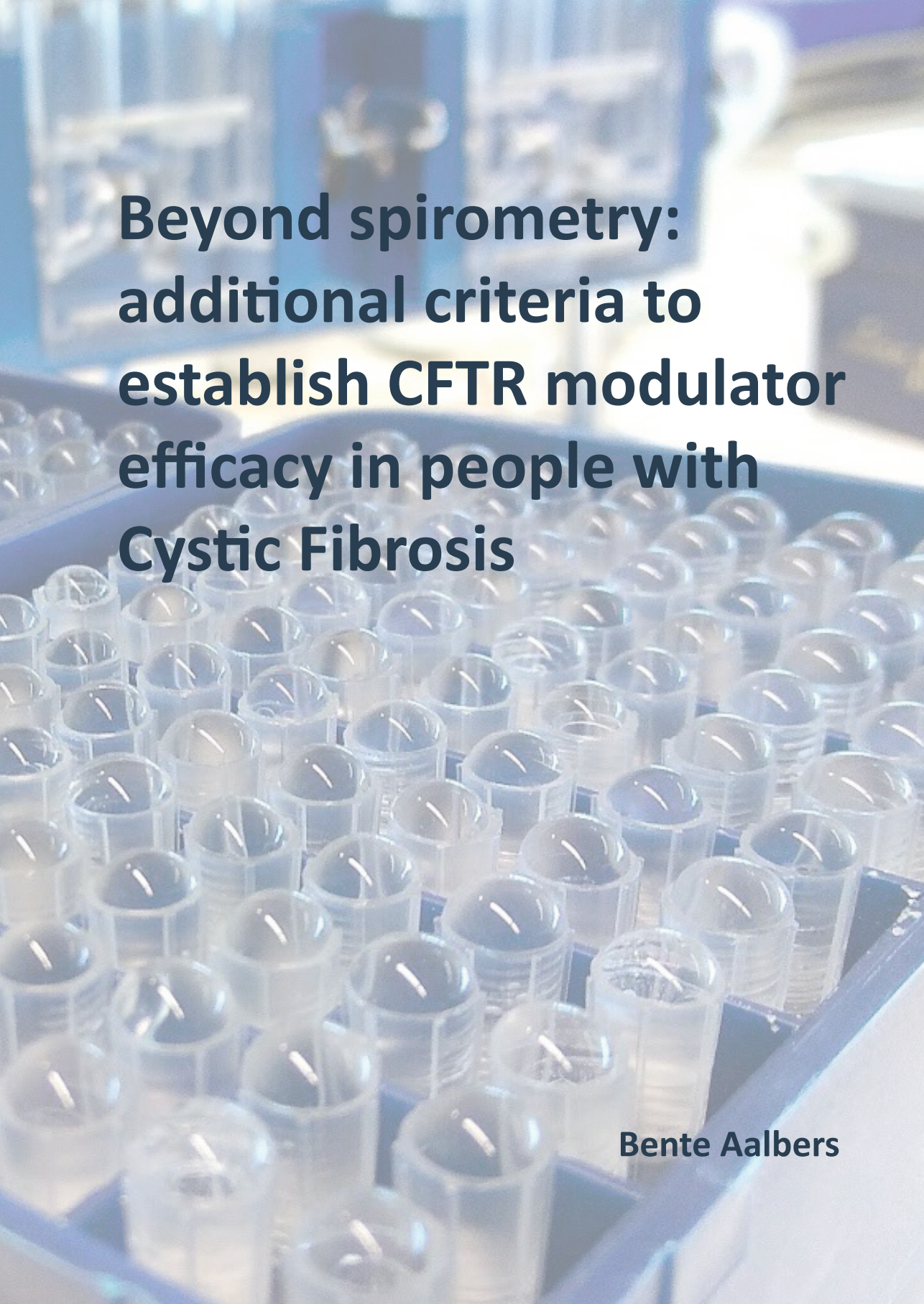

Beyond spirometry: additional criteria to establish CFTR modulator efficacy in people with Cystic Fibrosis

Bente Aalbers

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Meer dan spirometrie alleen: aanvullende criteria voor het aantonen van CFTR modulator effectiviteit bij mensen met CF

(met een samenvatting in het Nederlands)

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CHAPTER 1

General introduction

General introduction into Cystic Fibrosis, CFTR modulators and CFTR function assays

Cystic Fibrosis

Cystic Fibrosis (CF) is the most common life shortening autosomal recessive genetic disease, with an incidence of about 1:4750 births in the Netherlands. [1] It is caused by mutations in the *CF Transmembrane conductance Regulator (CFTR)* gene, which encodes a transmembrane chloride and bicarbonate channel expressed in epithelial cells throughout the body. [2]

Depending on the mutation in the *CFTR* gene, the resulting CFTR protein can be present in reduced amounts. It is either fully absent due to a lack of mRNA production (class Ia) or insufficient length of mRNA (class Ib), destructed by the cell before reaching the cell membrane (class II), present in reduced amounts (class V) or unstable (class VI). In other mutation classes there is a normal amount of protein, but with reduced opening probability (class III) or reduced channel function (class IV). While this division into classes helps to understand mutation effects, it is an underrepresentation of the true complexity, as most mutations share characteristics of multiple classes. [3]

Figure 1. CFTR mutation classes with examples and possible corrective therapies. [3]

	Normal	Class Ia	Class Ib	Class II	Class III	Class IV	Class V	Class VI
CFTR defect		No mRNA	No functional CFTR protein	CFTR trafficking defect	Defective channel regulation	Decreased channel conductance	Reduced synthesis of CFTR	Decreased CFTR stability
Mutation examples		Dele2,3 (21kb), 1717-1G->A	G542X, R533X, W1282X	F508del, N1303K, M1101K, G85E	G551D, S549N G1349D	R117H, R334W, R347P	3849+10kbC->T, 2789+5G->A, A445	rF508del, Q1411X
Corrective therapy		unrescuable	read-through, NMD inhbitor, correctors and potentiators	amplifier, correctors and potentiators	potentiators	potentiators	antisense oligonucleotides, correctors and potentiators	stabilisers

This insufficient CFTR protein function results in strongly reduced chloride and bicarbonate transport, and thus the epithelial lining becomes less salty. Subsequently, the mucus layer is left too dry with thick mucus as the salt should have attracted water,

and with disturbances in its pH. [4] The epithelium is then vulnerable to infections and prone to obstruction by accumulating thick mucus. This causes problems in the airways, but also the intestines, pancreas, liver, reproductive tract and sweat glands.

CF is a multi-organ disease as most epithelia are affected. Since malnourishment as a result of pancreatic insufficiency had become manageable by the administration of pancreatic enzyme suppletion, it has been mainly considered a lung disease as the problems with the airways and lung tissue for most patients are the most severe and life-shortening.

Because CF-related health problems can have a very early onset in life and involve intensive therapies with frequent hospital visits, the psychosocial impact of the disease on patients, family members, friends and peers can be severe. This is reflected in the yearly psychosocial evaluations and quality of life questionnaires completed in the CF centers.

CFTR modulators

For an increasing number of CFTR mutations, it is possible to target the aberrant CFTR protein using small molecule therapies that improve its function and (partially) restore chloride, bicarbonate and fluid transport. These small molecules are called CFTR modulators. These can be divided into two groups: CFTR potentiators and CFTR correctors.

CFTR modulators are small molecules that bind directly to the CFTR protein, altering its conformation and thereby changing its functional properties. [5] They were initially developed to target F508del CFTR protein, but were later repositioned for other CFTR mutations when effects on these mutations were established in vitro. [6] [7]

CFTR modulators are the first class of drugs that directly target the CFTR protein, thus acting early in the cascade of events that starts with the genetic defect causing insufficient CFTR protein. Before the advent of CFTR modulators, all treatments for CF were based on managing complications of the CFTR protein insufficiency, rather than restoring protein function. Because CFTR modulators partially restore this function as an ion channel, it can prevent CF-associated complications and subsequent structural damage. This potential to alter the course of the disease makes these drugs revolutionary in CF treatment. Because the protein is targeted which is subject to turnover in the cell, continuous use of the modulators is essential for sustained effects. Therefore, although these drugs are highly effective, they do not constitute a cure.

CFTR potentiators

CFTR potentiators increase the open probability of the anion channel, allowing more ion transport across the cell membrane [8]. They can be used to treat individuals who carry class III (gating) mutations that result in sufficient protein at the apical surface but fail to adequately open to ensure sufficient chloride and bicarbonate transport. By acting on surface-localized CFTR, potentiators have immediate effects on CFTR function. In some mutations primarily classified as class IV and V, potentiators are also effective, and a clear relation between residual function and ivacaftor efficacy has been observed in vitro (REF Dekkers Science TR Med 2016).

CFTR correctors

CFTR correctors increase mutant protein trafficking towards the apical cell membrane by binding to the protein, altering its folding and thereby protecting it from endoplasmic reticulum associated degradation. Correctors thus increase the number of CFTR channels present on the cell membrane, leading to increased function when the corrected protein can be functionally active. Many corrected mutant CFTR proteins including F508del further benefit from CFTR potentiator co-treatment that help to open the corrected CFTR channel in response to cellular signaling. Examples of clinically used correctors are lumacaftor, tezacaftor and elexacaftor, the latter being unique for having both corrector and potentiator activity. [9]

Combined CFTR modulator therapy

Monotherapy with a CFTR potentiator (ivacaftor) is only effective in people with a class III, IV or V mutation. In order to improve CFTR function in class II and some class V mutations, a combination of a CFTR potentiator and one or two correctors is needed. So far, available combination drugs are ivacaftor/lumacaftor, ivacaftor/tezacaftor and ivacaftor/tezacaftor/elexacaftor.

Opportunities and challenges resulting from CFTR modulator therapy

Highly effective treatment in specific CFTR genotypes

Efficacy on the short term has been established for single, dual and triple CFTR modulating treatment in the context of defined CFTR genotypes. The first successful phase III trial for a CFTR modulator studied ivacaftor in people with CF (pwCF) with at least one G551D mutation and found a mean change in $ppFEV_1$ of +10.6% and a mean change in sweat chloride concentration (SCC) of -48.1mmol/l [4,8]. In the phase III trial for lumacaftor/ivacaftor in pwCF with F508del homozygous genotype, mean difference in $ppFEV_1$ between treatment and placebo was 2.6-4.0% after 24 weeks, while exacerbation rate was 30-39% lower in the treatment group [10]. Phase III trials with tezacaftor/ivacaftor show similar results but with less side effects [11]. The phase III trial for elexacaftor/tezacaftor/ivacaftor showed a larger treatment response: after

28days, difference in ppFEV$_1$ change was 10.0 and difference in sweat test response was -45.1mmol/l compared to treatment with tezacaftor/ivacaftor in patients homozygous for F508del. For people with one F508del allele, mean difference in ppFEV$_1$ was 13.8 and difference in sweat chloride was 41.8mmol/l between treatment and placebo after 28 days. [12, 13]

From earlier literature we know that the rate of lung function decline is correlated to the prognosis in pwCF. It is therefore expected that these drugs, that influence lung function and lung function decline, also are of major influence on the life expectancy of patients with CF. A study using a prediction model based on established prognostic factors in CF shows an estimated mean life expectancy of 71 years for F508del patients using ELE/TEZ/IVA. [14] Availability of the CFTR modulators has been too recent to confirm this in trials. It has also proven to be difficult to predict long-term effects of LUM/IVA on lung function decline based on short term effects. [15]

From the available evidence, we can conclude that effective restoration of CFTR function has clinical consequences that are expected to have an important impact on both the daily functioning and prognosis of pwCF.

Assessment of efficacy

As shown above, treatment efficacy for CFTR modulators in clinical trials can be measured on a population scale, but it remains unclear how to translate the observations from these trials to individuals with CF. One complexity is the progressive nature of CF, resulting in challenges how such progression can be predicted by short term observations at the level of the individual, and whether progression can be halted or established disease even be reversed.

Another challenge that these new therapies present, is determining how the long term effectivity of these drugs should be measured and if this can be predicted using short-term outcomes on an individual basis. The magnitude of effects will differ tremendously from patient to patient, even if they have the same genotype. In addition, observations also show differences in which organ systems respond well to the CFTR modulators and which do not. Variations are seen in responses between sinonasal symptoms, gastro-intestinal symptoms, pulmonary symptoms and sweat chloride. As CF has a progressive disease burden over time, the main effect of treatment should be stability in the long term. It would be preferable to find short term outcome measurements that predict this long-term stability.

To achieve this, a composite score would logically be required, involving at least the improvement in CFTR function and the extent of irreversible organ damage. Development of such a composite score has so far not been carried out. It is beyond the scope of this thesis and might probably not be possible. It is not expected that a

complete and accurate prediction will be feasible on an individual level, as various factors influencing prognosis are unpredictable, e.g. future comorbidities, patient lifestyle and support system over time, side effects of therapy. Even so, any attempt to estimate individual treatment effect of CFTR modulators will include CFTR function measurement as a key parameter as well as various clinical parameters.

In this light, we needed to explore which follow up parameters best represent the relevant effects of modulator drugs for the individual. This called for an integrated approach where clinical status was assessed using various modalities such as FEV_1, BMI, CT scan, sweat test and CFQ. Where needed, these were complemented by lab-based relevant disease models, like cultured nasal epithelial cells or intestinal organoids, which are either patient-specific of genotype-specific.

Appropriate use

Another important challenge is to ensure appropriate and efficient use of these costly drugs. In the context of this thesis, appropriate use means that all patients who will definitely benefit from the drug should receive it, and no patients should be treated with it when they have no benefit or experience unacceptable side effects. It will be important to identify better criteria for start and stop of treatment. Criteria for start are now usually genotype and age based. For patients with genotypes that are not yet identified as eligible for starting the drugs but might benefit, and for patients with genotypes with known varying responses to treatment, effectivity assays in cell models such as organoids may provide a better basis to decide on eligibility compared to genotype alone.[16]

Notably, there are differences between the list of genotypes approved by the FDA (based on FRT cells as a readout for CFTR function) and EMA (at least one F508del mutation must be present). For patients with a genotype which is not on the applicable list, there is a need for an outcome measurement that reveals if a patient will be responsive to new treatments, based on a short clinical treatment trial or in vitro assay.

At this moment, criteria for start of the drugs are usually well-defined and based on patients' genotypes, but these leave out patients with other genotypes that may benefit from the treatment as well. Moreover, no widely accepted criteria are available for discontinuation of treatment due to ineffectiveness. In current clinical practice, the only reason for discontinuation of the treatment is due to side effects or patient preference.

Along with the opportunities that the new CFTR modulators bring to patients and their caregivers, due to a more stable condition over time, come certain challenges concerning the adaptation of CF care to this new situation. Less intensive follow up and care may be needed.

CFTR functional assays used in CF diagnostics and treatment follow up

The CFTR protein and its function as an anion (chloride and bicarbonate) channel are key in the application of CFTR function assays. Several modalities for the assessment of CFTR function were originally used for diagnostic purposes. With the rise of CFTR modulator drugs, many of these were repurposed for assessment of treatment effects, in response to the need for adequate effect markers for CFTR modulators. As treatment response can differ between various tissues within the body and across individuals (even with the same genotype), multiple assays should be used to evaluate drug response. There is an ongoing search for the best combination of outcome measurements and for cutoff values for a clinically relevant response. The most important CFTR function assays will be discussed below.

Sweat test

Sweat chloride concentration (SCC) is measured in sweat which is collected in a standardized manner with a minimum sweat rate of 1g/m^2/min, after application of transdermal pilocarpine by iontophoresis. The sweat is subsequently collected in filtration paper, gauze or a small caliber duct, and analyzed by argentometry. [14] For diagnostic application of the test, normal range is <30 mmol/l, borderline values are 30-60 mmol/l and diagnostic for CF is >60 mmol/l. For patients with well-classified genotypes, sweat chloride was not an independent predictor for survival. Among patients with unclassified CFTR genotypes, sweat chloride is correlated with survival. [20]

Because it is a feasible and repeatable in vivo readout for CFTR function in people of any age, it is also widely used in the follow up of CF patients starting CFTR modulating treatment. When the test is used for follow up, values before start of treatment are compared to the values during treatment, without absolute cut-off values. No minimal clinically important difference (MCID) is reported in literature. In clinical trials, sweat test is used as a secondary outcome measurement, showing drastically improved chloride values after treatment with IVA or ELE/TEZ/IVA. On an individual level, no correlation between change in sweat chloride and FEV_1 has been established [8, 12, 13]. In the future, when CFTR modulating treatment will be initiated in infants instead of older children and adults, sweat chloride will probably become a main outcome measure as FEV_1 cannot yet be measured at such a young age. Sweat test is a feasible method from birth onward, and a parameter that is correlated to disease progression over time.

Nasal potential difference (NPD)

NPD test measures the potential difference (PD) between a fluid-filled exploring bridge (double lumen catheter) affixed on the nasal mucosa and a reference bridge most frequently inserted into the subcutaneous space of the forearm. Both bridges

are linked by electrodes to a high-impedance, low resistance voltmeter amplifier. The exploring catheter is advanced under the inferior turbinate and PD is recorded along the nasal mucosa. It is then positioned at the point with the most negative signal. Basal potential difference (baseline PD) is measured after perfusion of Ringer's saline solution representing the maximally negative, most polarized, stable baseline PD value (PDmax). Subsequently, PD changes are recorded after the following superfusions: 100 µmol amiloride in Ringer's saline solution (PD change is referred to as Δ amil), 100 µmol amiloride in chloride (Cl^-)-free solution to drive CFTR-mediated chloride secretion (Δ Clfree), 10 µmol isoproterenol in the chloride-free solution to stimulate cAMP-dependent CFTR-related Cl^- transport (Δ Iso). The sum of the two responses (Δ Clfree+Iso) serves as an index of CFTR function. Adenosine triphosphate (ATP) is superfused at the end of the test as a positive control. Each solution is perfused at room temperature at 5 ml/min (300 ml/h) for a minimum of three minutes for amiloride and isoproterenol and for five minutes for chloride free solution [21, 22, 23]. Solutions are changed as soon as a steady, noise-free voltage tracing is achieved, and the differences in NPD values are measured between the plateaus of the corresponding solutions. The test is performed in both nostrils.

This approach to in vivo measurement of CFTR function allows for a real-time readout, and it is mainly used for diagnostics, if sweat test and genetic analysis do not provide a clear confirmation or rejection of a CF diagnosis, and assessment of CFTR function in addition to sweat test is needed. In addition, in recent years it has also been used as an outcome parameter in clinical studies involving CFTR modulators. [8, 9] It provides a real-time in vivo estimation of CFTR function in a disease-relevant tissue, however the measurement itself and preparation of the setup are time-consuming and extensive training for study personnel is essential. The serial measurements that are needed for use as a follow up parameter therefore make this approach labor intensive.

Intestinal current measurement (ICM)

Intestinal current measurement (ICM) is an ex vivo electrophysiological technique to measure CFTR function in rectal biopsies, as CFTR is highly expressed in the rectum. Ussing chambers record the transepithelial short-circuit current (Isc) as a measure of ion transport while it is stimulated with chloride-secretory agents. The amount of residual function of the CFTR chloride-channel can be assessed. [24]

The rectal biopsies are 2-3mm and after obtaining them they are transported to the laboratory in ice cold phosphate-buffered saline and directly placed into the Ussing chamber. In these chambers, oxygenation and recirculation of the serosal and mucosal bath fluids are maintained by airlift pumps with a composition of the bath fluid with a glucose containing Meyler buffer solution at 37°C and gassed with 95% O_2 and 5% CO_2. The tissue is short-circuited by voltage-clamp which results in a continuously recording of the Isc and in a zero transepithelial potential difference. The basal transepithelial

resistance and basal potential difference (in open circuit) are also measured. The Isc shows the net movement of ions across the epithelium after adding specific agents to the buffer solution on the mucosal and serosal side. Each biopsy is subsequently exposed to multiple secretagogues. Subsequently, additions consist of amiloride, forskolin/IBMX, genistein, carbachol, DIDS, histamine. [32, 33, 34]. The tissue of the rectum can be a target organ of disease that is not altered by CF manifestations or progression. The rectum biopsies are studied ex vivo, which provides an opportunity to add agonists that can be used to detect and quantify CFTR activity. [35, 36, 33] In the diagnostic workup for CF, the responses to carbachol and IBMX/forskolin can be used to quantify CFTR function, which is used in cases when sweat test is borderline and genetic analysis is not conclusive. As such, ICM is part of the Dutch CF care protocol. [30] However, with the development of in vitro techniques requiring less specific training of personnel, for which correlation with clinical parameters was also established, diagnostic ICM is mainly performed in a research setting. In specific laboratories, ICM is also used for the ex vivo assessment of therapeutic effects, mainly concerning potentiators. [31, 32] For this purpose it still remains a labor-intensive modality compared to other available techniques.

Forskolin induced swelling (FIS) assay in organoids

Patient-derived intestinal organoids facilitate the measurement of individual CFTR function through the forskolin-induced swelling (FIS) assay, enabling both the assessment of CFTR residual function and the effects of CFTR modulators. This assay measures CFTR-dependent ion and fluid transport into the organoid lumen that causes rapid organoid swelling.

Biopsies from the intestinal mucosa are taken from the individual patient and stored in ice cold PBS for transfer to the lab. Crypt cells are isolated from the biopsies and left to proliferate, spontaneously forming droplets of cells, consisting of a closed epithelial cell monolayer with a central lumen. These droplets are described as organoids. As the cells proliferate further, these organoids grow and are disrupted to form new smaller organoids. After a few of these proliferation steps, organoids can be stored in a biobank with the patient's consent for later use in research and for further proliferation, or they can directly be used for FIS assay. Culturing of the intestinal crypt cells for organoids can still be done after ICM has been performed on the biopsies, albeit that viability of the tissue is lowered. To perform FIS assays, organoids are dispersed in a 96-wells plate, pretreated with CFTR modulators if indicated, stained with calcein green and imaged by time-lapse microscopy. Swelling of the organoids is recorded after various concentrations of forskolin are added, by imaging for 60 minutes at 10 minutes intervals. This swelling is usually reported as the AUC, using the curve of the relative swelling (percentage area increase compared to T = 0) as a function of the time, presenting AUC per forskolin concentration. [33, 34]

Figure 2. FIS in organoids derived from F508del/F508del CF patient cells with and without CFTR modulators, compared to healthy control [25]

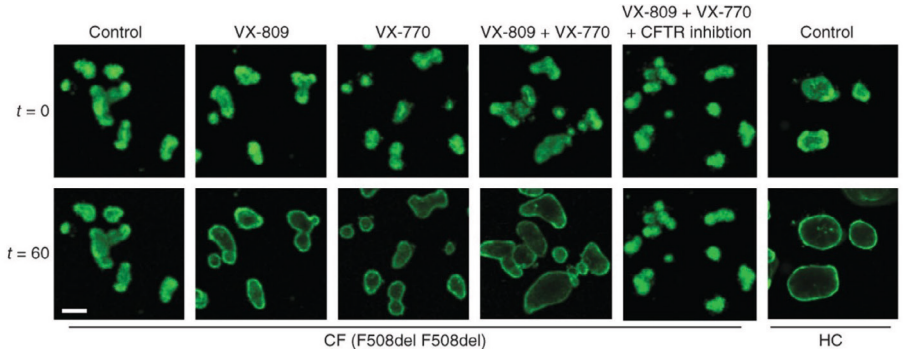
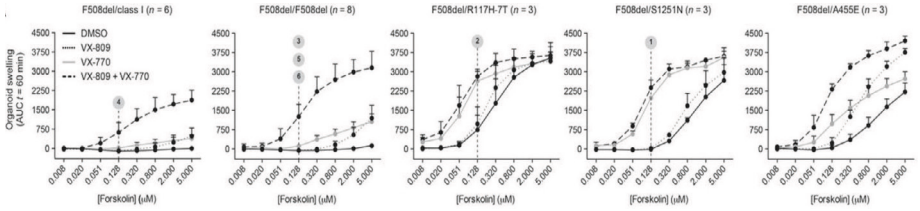


Figure 3. AUC curves for FIS assay with VX-770, VX-809 and combination compared to vehicle only in organoids with various CF genotypes (dotted line: forskolin concentration used for correlation with clinical data) [25]



To have an in vitro CFTR functional readout for airway cells, roughly the same technique can be used for nasal cells or bronchial cells acquired though mucosal brushing. However more cell culturing steps are needed to select the basal cells and help them to keep replicating, compared to the intestinal stem cells. To use this technique, cells are first cultured as a 2D layer and then disrupted to form spheres to be able to perform a FIS assay. [27]

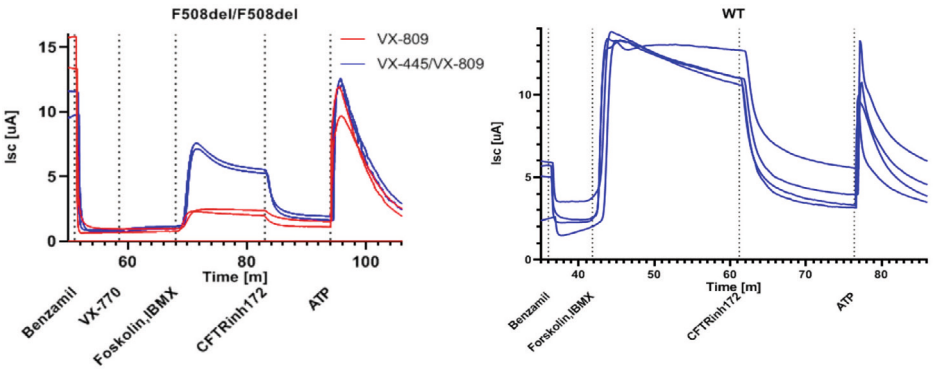
2D cell layers in Ussing chambers

A complementary, labor intensive but direct in vitro assay for CFTR function, is measurement of current through cell monolayers after culture on transwell filters.

When measuring airway cells in this manner, cells are first air exposed for multiple weeks creating an air-liquid interface (ALI), to allow differentiation into the mature airway cell types that express CFTR. The differentiated cells can be measured in Ussing chambers, if needed after pretreatment with possible therapeutic compounds. The chamber compartments are filled with buffers with a chloride gradient to support chloride transport. After placement of the filters and buffer and reaching a stable current, secretagogues are added in a similar but not identical manner compared to ICM. Benzamil, forskolin/IBMX (and if needed, a CFTR potentiator), CFTR inhibitor and ATP are added subsequently, respectively to block ENaC, stimulate CFTR function, inhibit CFTR function, and assess calcium mediated anion transport.

These assays in intestinal and airway cells provide a patient-specific readout for CFTR function in a diagnostic and prognostic setting and for in vitro prediction of clinical CFTR modulator treatment effects. Apart from the culturing conditions necessary, there are two main differences between the intestinal and airway cells: Firstly, in intestinal epithelium forskolin induced chloride secretion is fully CFTR dependent. In airway epithelium, forskolin induced chloride secretion is not fully CFTR dependent as the cells also express alternative chloride channels (such as TMEM16A). As such, additional inhibitors are used to pinpoint the CFTR dependent current in airway cell models. Second, the intestinal stem cells express more CFTR compared to the differentiated airway cells. These two factors explain why the applicability of the assays can be different between the cell types: the forskolin induced swelling assay in intestinal organoids is very sensitive to CFTR activity, even in low amounts, while it cannot discriminate between moderate and very good CFTR function, as the swelling is limited. To assess CFTR function in the higher ranges, other assays can be performed in intestinal organoids without forskolin stimulation, e.g. steady state lumen area (SLA) or rectal organoid morphology assay (ROMA) [36, 37]. This limitation of the FIS is less likely to be restrictive in airway organoids due to the lower expression of CFTR, and is not present in airway cells on filter, as this assay does not rely on swelling as a readout, although in this assay the basolateral influx of chloride can limit CFTR based chloride transport through the apical cell membrane. For smaller differences in CFTR function, the airway cells will be less sensitive. The presence of other chloride channels on the airway cells can be both a challenge and an opportunity; it is a necessity to block the alternative chloride channels to have a CFTR specific readout, however, it does make the airway cultures a suitable model for the exploration of therapeutic options that stimulate these alternative chloride channels in order to restore anion transport. [38]

Figure 4. Ussing tracings of ALI cultured nasal cells of CF patients with F508del/F508del genotype, treated with VX770+809 or VX770+809+445, compared to wildtype (WT)



Clinical parameters in follow up of CF patients on CFTR modulators

As CF affects various tissues throughout the body with varying severity between patients, it would be inadequate to use only one clinical parameter for evaluation or treatment effect of CFTR modulators. Realistically, a combination of CFTR function testing and clinical assessments in different tissues as well as general parameters (such as BMI as an estimate for nutritional status) should be used. The main clinical parameters that have been used in this thesis are discussed below. This is not a complete list of possibly relevant outcomes. For example, assessment of sputum cultures over time, resting energy expenditure and exercise testing may also be relevant but do not have a central role in this thesis.

Spirometry: FEV_1

Forced expiratory volume in one second (FEV_1) is assessed through spirometry: A patient is asked to breathe through a mouthpiece while the nose is closed. Next, the patient performs a maneuver by starting with maximal inspiration followed by forced and maximal expiration through this mouthpiece, so that the flow is recorded. From this flow FEV_1 can be calculated, and is usually reported as $ppFEV_1$; percentage of predicted for FEV_1 based on reference data of healthy controls, based on height and age. It serves mainly as a measure for airway obstruction but is also influenced by lung volume. FEV_1 is a well-established follow-up parameter in standard CF care and has an established correlation with prognosis. It is therefore used as a primary outcome measure in the phase III clinical trials for CFTR modulators, to establish clinically relevant responses in different genotypes with various CFTR modulating treatments. [12, 13]

In a study focused on predicting factors for the long term outcomes of CFTR modulating therapy, acute change in FEV_1 as well as the rate of FEV_1 decline after start of treatment

was not associated with any of the candidate predictors on an individual level. [15] This confirms that FEV_1 alone is not sufficient for evaluating if the treatment response to CFTR modulators is affecting prognosis of an individual with CF.

CT thorax (Brody score)

Computed tomography scan of the chest is a fast way to visualize the structural changes in the lungs caused by CF. Scoring of these changes according to Brody is divided in subdomains: bronchiectasis, mucus plugging, peribronchial thickening, parenchyma (opacities, ground glass, cysts) and air trapping. Each of these domains is scored per lobe by a radiologist, creating numeral subscores per domain and an overall score on a scale from 0 to 40.5 per lobe. This overall score and subscores represent the severity of structural changes in the lungs and can be used in follow up over time. Brody score has been validated for this purpose in both pediatric and adult CF patients. [39]

A disadvantage of using CT thorax is the use of radiation. Although the dose that is used per scan is low (5.6 mSv), it is a reason to be restrictive in the frequency of performing these scans to reduce the cumulative lifetime radiation dose for patients. However, it provides more information compared to X-thorax. CT thorax is also is better validated, less costly and faster compared to MRI of the lungs, which has the advantage of avoiding ionizing radiation. [40]

In daily practice, CT thorax is not standard of care for follow up in every CF clinic. It is performed in individual patients to evaluate possible causes of clinical deterioration, such as pulmonary embolism or fungal infections. So far it has not regularly been used as an outcome measure in clinical trials. We have explored its use for this purpose in chapter 8.

CFQ-R

The CF questionnaire (CFQ), which was replaced in 2018 by a revised version (CFQ-R), is a standardized and CF specific questionnaire on health-related quality of life. It consists of 14 domains: physical functioning, role functioning, vitality, health perceptions, emotional functioning, social functioning, body image, eating disturbances, treatment burden, weight, respiratory symptoms, and digestive symptoms. Each domain is standardized on a 0-100 score, higher scores indicate better quality of life. The CFQ-R has been translated in various languages (among which Dutch) and has been validated in CF patients. There are separate versions for teens/adults and for children and their parents or caregivers. [41, 42, 43]

This questionnaire can be repeated periodically in follow up to monitor health-related quality of life, and is also used in clinical trials for CFTR modulating treatment. [44] However, despite standardization, the minimal clinically important difference has only

been established for the respiratory domain of the CFQ. Therefore, a change in score in other domains is difficult to interpret. [45]

Aim of this thesis

The main aim of this thesis is to explore which CFTR functional assays and clinical parameters can help to evaluate effects of CFTR modulating treatment, as FEV_1 is useful as an outcome parameter on a group level in clinical studies, but monitoring FEV_1 alone is insufficient for evaluation of treatment effects in individual patients.

Previous studies have shown that acute FEV_1 response to treatment is not associated with prognosis and long-term FEV_1. In the clinical studies, FEV_1 and other clinical outcome parameters such as sweat chloride, BMI and pulmonary exacerbation rate were not mutually correlated. [15] CFTR genotypes appeared to be a useful predictive factor at the group level, but they are not predictive of treatment effect at the individual level. [46] As such, we need better combinations of outcome parameters to estimate treatment effects on the individual level. This thesis is aimed to contribute to this by exploring various outcome measures.

This aim can be divided into two perspectives:

1. Using CFTR functional assessment to establish restored CFTR function in patients' airway cells. These assays can be of use if estimation of effectivity is needed before start of treatment, for example if the indication is uncertain and thus modulators are not reimbursed or available through managed access programs.
2. Using clinical parameters to monitor treatment effects in patients. This is mainly important in situations where FEV_1, which is the most widely used parameter, is less indicative of treatment effects, due to extensive structural damage or particularly well-preserved lung function.

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PART I
CFTR functional assays

CHAPTER 2

Nasal potential difference in suspected cystic fibrosis patients with 5T polymorphism

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ABSTRACT

Background

5T polymorphism is a CFTR mutation with unclear clinical consequences: the phenotype varies from healthy individuals to Cystic Fibrosis (CF). The aim of this study was to evaluate if nasal potential difference (NPD) and sweat testing correlate with symptoms and CF diagnosis in 5T patients.

Methods

86 patients with 5T who had undergone NPD measurement, were included (6 homozygous (5T/5T), 41 with a PI-CF causing mutation in trans (5T/PI-CF), 11 with a PS-CF causing mutation in trans (5T/PS-CF) and 28 without a known mutation in trans (5T/?). Data including age, phenotype, sweat chloride and follow up were collected.

Results

33% of the 5T/5T patients had abnormal NPD results, compared to 70% in 5T/PI-CF; 33% in 5T/PS-CF and 29% in 5T/?. The percentage of high or borderline sweat chloride was highest in 5T/PI-CF, and 5T/?, compared to 5T/5T and 5T/PS-CF (91, 96, 80, and 63%, respectively). TGm (number of TG repeats in intron 8) analysis was performed in 21 5T/PI-CF patients. TG11 was associated with lower sweat chloride, lower percentage of abnormal NPD and less progression of symptoms compared to TG12 and TG13.

Conclusion

There is much variation in clinical status among 5T patients. All patients in this study with 5T/PS CF, all patients with both normal NPD and sweat test, and most patients with TG11 were stable or improving over time. Therefore, NPD measurement and TGm status aid to assess if a patient is at high risk for developing CF or CFTR-related disease and if specific follow up in a CF center is required.

Introduction

Cystic Fibrosis (CF) is the most common life-limiting autosomal recessive inherited disorder. It is caused by mutations in the CFTR gene, leading to inadequate function of the CFTR protein [1]. Over 2000 different mutations have been discovered since 1989 [2]. Different types of mutations seem to correlate with different grades of residual CFTR function, so that certain types of mutations lead to more severe disease than others [3]. Throughout this article 5T polymorphism is referred to as 5T, but in literature it is also called IVS8-5T or c.1210-12T [5]. This polymorphism occurs in the polythymidine tract in intron 8 of the CFTR gene, leading to inappropriate splicing whereby exon 9 is left out in 70–90% of the mRNA strands, resulting in a CFTR protein that will not reach the cell membrane. The other intron 8 polythymidine tract polymorphisms 7T and 9T lead to 0–50% and <5% aberrant transcript, respectively [4,5].

When combined with a CF causing mutation on the other allele and R117H in cis, 5T is known to cause CF by aggravating the effect of the R117H mutation [6]. If present solely on one allele and combined with a CF causing mutation in trans (compound heterozygous), 5T may have various consequences, from no symptoms or isolated CBAVD to pancreatic sufficient CF (PS-CF) or even pancreatic insufficient CF (PI-CF) [7,8]. The extent of correct splicing of exon 9 is further determined by the number of adjacent TG repeats (TGm), located upstream of the Tn locus of intron 8. The number of TG repeats in cis on a 5T background influences the efficiency of splicing, hereby acting as a complex allele [9]. According to the CFTR2 database, 5T (not in a complex allele) is defined as a 'mutation with varying consequences', irrespective of the number of TG repeats; individuals who are homozygous for 5T – whether TG11, TG12 or TG13, without R117H – have all been reported across the full spectrum of CFTR disorders from healthy individuals to CF, although symptoms are usually mild in the majority [10,11].

Patients that are compound heterozygous for this mutation may be symptomatic and suffer from a mono-organ disease, e.g. CBAVD (congenital bilateral absence of vas deferens), sinusitis or bronchiectasis, and some have multi-system disease and fulfill the diagnosis for CF.

5T variant is particularly common in Ashkenazi Jews and accounts for 18% of all PS CF cases; in non-Ashkenazi Jews this is 10%. 32% of CBAVD in Ashkenazi Jews is associated with 5T polymorphism, and 36% in non-Ashkenazi Jews [12]. 5T is also widely present in other ethnic groups [13,14].

This study aims both to provide descriptive information about a large cohort of 5T patients referred for diagnostic workup, and to evaluate if NPD measurement is of added value in these patients to estimate CF severity and the need for follow up.

Methods

Patient characteristics and follow up data

Data was collected from patients with at least one 5T allele, who were seen in a CF center for diagnostic evaluation, in a retrospective manner. This includes patients with bronchiectasis, recurrent pancreatitis, CBAVD, or in case of a family history of CF. Tables 1.a and 1.b provide an overview of the nature and extent of symptoms leading to referral. Patients were included if they underwent at least genetic testing of both alleles (in a panel or full sequencing), NPD and sweat test. In all patients, information about age, gender and CFTR mutations on both alleles was collected. These mutations were grouped in PI and PS CF causing mutations, according to the CFTR2 database [10] (Table 2). Mutations with varying or completely unknown clinical consequences were also included in the group with PS CF causing mutations. In 38 patients information about the TGm status was available.

Other variables collected were symptoms at the time of evaluation and follow-up of these symptoms during at least one year(median follow up duration: 5.0 years, range 1.1–26.0 years), as well as the final diagnostic state after full evaluation (CF, CFTR-RD, no CF), according to the clinician. Ethical approval for the anonymous use of patient data for research was ensured in all participating centers.

Table 1a. Frequency of symptoms contributing to referral to a CF center

		Number of patients (%)
Respiratory	Bronchiectasis or recurrent lower respiratory tract infections	36 (42%)
	Obstructive lung disease (including astma/COPD)	15 (17%)
	Sinusitis and/or nasal polyps	21 (24%)
	Chronic cough/sputum	9 (10%)
Gastro-intestinal	Recurrent pancreatitis	10 (12%)
	Pancreatic insufficiency/chronic diarrhea/failure to thrive	11 (13%)
	PSC/liver test abnormalities/cholelithiasis	4 (5%)
CBAVD		16 (19%)[a]

[a] 19% of total, 29% of male patients

Table 1b. Extent of symptoms per patient

	Number of patients (%)
No symptoms, family history led to referral	7 (8%)
Symptoms in one organ	49 (57%)
Symptoms in multiple organs	30 (35%)

Sweat chloride

Sweat test was performed in a standardized manner in certified laboratories. A minimum of 1 g/m^2/min was collected for 20–30 min by iontophoresis, after application of pilocarpine on the skin. Chloride con- centration was measured in the collected sweat, according to the CFF guidelines [15]. Most patients had multiple (2–4) sweat tests taken. In these cases the mean result was used for the study. In seven patients no sweat test results were available, for five patients this was due to repeatedly insufficient sweat volume obtained on multiple occasions. One patient suffered from ichthyosis, and one patient refused to undergo a repeat test after the first attempt yielded insufficient sweat volume.

Nasal Potential Difference (NPD)

NPD test was carried out in all centers according to the Standardized Operating Procedure of the ECFS [16]. Each operator had received training in the technique before the commencement of the measurements.

NPD measurement data was obtained retrospectively from multinational CF diagnostic centers (Jerusalem, Berlin, London, Leuven, Brussels, Hannover, Utrecht), where NPD measurements were performed in the patients for diagnostic purposes. Subsequently the data was anonymized and assembled in a data file. The response to amiloride (Δamil) and the response to chloride free solution added to the response to isoproterenol (ΔClfree+Δiso) was derived. From this, the exponent of (Δamil/(ΔClfree+Δiso)) was calculated as a measure for CFTR function. An $e^{(\Delta amil/(\Delta Clfree+\Delta iso))}$ of 0.7 or higher indicates a poor CFTR function as is seen in CF, being defined as an abnormal NPD result [17,18]. All centers followed the SOP as provided by the ECFS; therefore the only difference noted between the centers was the way the reference electrode was attached to the skin. In most centers a subcutaneous needle was inserted into the forearm, in a few centers the skin abrasion technique was used, where a superficial skin abrasion is made and the electrode was attached onto the skin. Both methods are described in the SOP and shown to discriminate CF from non-CF subjects in a comparable way.

Approach to description of 5T patients and subgroups

The patients were divided into four groups according to the mutation on the second allele:

Group 1: Homozygous for 5T
Group 2: Mutation in trans known to cause PI CF (Table 2)
Group 3: Mutation in trans known to cause PS CF or with various clinical consequences
Group 4: no second mutation found.

The patients with an abnormal NPD result ($e^{(\Delta amil/(\Delta Clfree+\Delta iso))} \geq 0.7$) were compared to the patients with a normal NPD result.

Patients were also divided according to their symptom evolvement during follow up; either their symptoms resolved or remained stable over time, or they showed progressive or recurrent symptoms during follow up. If patients suffered from bronchiectasis, sinusitis or CBAVD and had no new symptoms or recurrent infections, they were classified as stable. If there were recurrent infections or patients suffered from recurrent pancreatitis with episodes during follow up, this was noted as recurrent symptoms. Any new symptoms or worsening of existing symptoms was also noted as clinical worsening.

CBAVD, acute recurrent or chronic pancreatitis and disseminated bronchiectasis as a mono-organ disease were defined as CFTR-RD [19]. CF diagnosis was recorded if the treating physician concluded that a person had CF based on multi-organ involvement with impaired CFTR function, after full evaluation [20].

Results

In total, 86 patients were included, of which 6 were 5T homozygous (group 1) and 52 were compound heterozygous 41 with a PI CF causing mutation in trans (group 2), 11 with a PS CF or varying consequence second mutation in trans (group 3; Table 2). In 28 patients no second mutation was found (group 4). In addition, we tried to obtain information about the length of the TG stretch (TGm) in cis in all patients, since research suggests that this has a major impact on splicing in 5T. TG repeats were known in 38 patients out of 86 (TG11: N = 7, TG12: N = 27, TG13: N = 4).

Table 2. PS CF and PI CF causing mutations *in trans* and their frequency in the studied group

PS CF causing (or varying/unknown clinical consequence) mutation (N=9)	PI CF causing mutation (N=43)
D1152H (c.3454G>C) N=4	F508del (c.1521_1523delCTT) N=28
F1052V (c.3154T>G)	W1282X (c.3846G>A) N=9
621+3A>G (c.489+3A>G)	G85E (c.254G>A) N=2
Q799K (c.2395C>A)	G542X (c.1624G>T)
N1303I (c.3908A>T)	1259insA (c.1127_1128insA)
V562L (c.1684G>C)	1717-1G>A (c.1585-1G>A)
	3056delGA (c.2924_2925delGA)

Legacy name is shown, with nomenclature according to HGVS (Human Genome Variation Society) between parenthesis.

When divided according to the mutations in trans, marked differences appear between the groups (Table 3). The percentage of patients with an abnormal sweat test is distinctively higher in group 2 and 4, compared to group 1 and 3 among which sweat test results were comparable.

The proportion of patients diagnosed with CF after full workup and follow up was 54% in the group with a PI CF causing mutation in trans, in the other groups this was 17–33%.

The percentage of males also differs between these groups. The difference may be partially due to small group size, although gender may also have an impact on CFTR function in 5T patients [21]. Males were frequently referred for diagnostic evaluation because of CBAVD as their main or only symptom, which is more common in the combination F508del/5T, accounting for 17% of the total of CBAVD patients [22]. This does not account for the full difference in this group.

When the patients with an abnormal sweat test were compared to those with a borderline and normal sweat test, those with a high sweat chloride (≥ 60 mEq/l) had surprisingly less worsening or recurrent symptoms (7%), compared to those with borderline (30–59 mEq/l, 31%) or low (<30 mEq/l, 33%) sweat chloride (Table 4). Patients with an abnormal sweat test were diagnosed with CF as often as patients with borderline sweat test (40% vs 43%), while none of the patients with low sweat chloride was diagnosed with CF.

Table 3. 5T patient characteristics according to mutations *in trans*

Division according to mutations	Group1: 5T/5T N=6	Group 2: 5T/PI CF N=43	Group 3: 5T/PS CF N=9	Group 4: 5T/? N=28
Mean age in years (range) n.s.	32 (16-63)	26 (1-69)	35 (8-76)	23 (7-65)
Gender (% male) n.s.	50%	74%	89%	61%
High sweat test	20%	17%	38%	29%
Borderline sweat test	60%	74%	25%	76%
Abnormal NPD	33%	70%*	33%	29%*
Worsening/recurrent symptoms n.s.	17%	21%	0%	4%
CF diagnosis	17%*	54%*	33%	17*
CFTR-RD diagnosis n.s.	17%	27%	22%	21%

n.s.: no significant differences between any of the groups.
Abnormal NPD: significant differences between group 2 and 4 (indicated with *, P=0.004).
CF diagnosis: significant differences between group 1, 2 and 4 (indicated with *, P=0.016).
CFTR-RD diagnosis: diagnosis defined by the CF center according to sweat test, NPD, mutations and clinical picture.

Table 4. 5T patient characteristics according to sweat test results

Division according to sweat test		Sweat chloride <30mEq/l N=9	Sweat chloride 30-60mEq/l N=52	Sweat chloride >60mEq/l N=18
Mean age in years (range) n.s.		35 (9-66)	27 (1-76)	23 (6-64)
Gender (% male) n.s.		75%	67%	88%
Abnormal NPD n.s.		50%	54%	59%
Worsening/recurrent symptoms n.s.		33%	31%	7%
Diagnosis n.s.	CF	0%	43%	40%
	CFTR-RD	0%	28%	7%
	none	100%	29%	53%

n.s.: no significant differences between any of the groups

When divided according to NPD results, it is noted that in the group with abnormal NPD results (exp ≥0.7) worsening or recurrent symptoms were more often seen compared to subjects with an abnormal sweat test. There also is a larger percentage of CF diagnoses and less patients that were categorized as not having CFTR-related symptoms, compared to the group with normal NPD. Frequency of CFTR-RD is comparable between the groups; 23 versus 20%, (Table 5).

When the patients are divided according to age at presentation, no important differences appear between the groups concerning sweat chloride, NPD results or progression of symptoms.

In the group of patients referred because of isolated CBAVD or without symptoms (N = 11), the percentage of patients with worsening symptoms over time was very low; only 1 patient had new symptoms in follow up. Three of the patients were diagnosed with CFTR-RD after full workup, none was diagnosed with CF. However, follow up time in this specific group was relatively short (mean: 3.9 years, range 1.1–14), so that new symptoms might have been missed in these patients.

TGm status was known in 38 of the patients. Within the group with known TGm repeats in cis, the groups with a PS CF causing (or varying consequences) mutation and without a mutation in trans, were too small to compare variables between groups with a different TGm state. In the group of patients with a PI CF mutation in trans (N = 26) a cautious comparison is possible however the groups are small: TG11= 4, TG12 = 18, TG13 = 4. The mutation in trans was F508del in all but 4 patients (1717 + 1G N A, 3056delGA, 1259insA and W1282X).

Fewer patients with TG11 had an abnormal NPD compared to those with TG12 and TG13. TG11 also was associated with lower sweat chloride, lower percentage of CF diagnoses and less worsening of symptoms over time (Table 6).

It is notable that two patient groups do particularly well over time. In these patients, none had worsening or recurrence of symptoms over time.

1. Patients with 5T and a PS CF causing mutation or a mutation with varying clinical consequence in trans (regardless of NPD and sweat test result).
2. 5T patients with both sweat chloride below 30 mEq/l and normal NPD results (regardless of the CFTR mutation in trans).

Table 5. 5T characteristics according to NPD results

Division according to NPD results		**Normal NPD (exp <0.7) N=44**	**Abnormal NPD (exp ≥0.7) N=42**
Mean age in years (range) n.s.		30 (3-69)	23 (1-76)
Gender (% male) n.s.		62%	79%
Mean sweat chloride (mEq/l, SD) n.s.		50 (18)	53 (16)
Groups	5T/5T	4 (9%)	2 (5%)
	5T/PI CF	13 (30%)*	13 (73%)*
	5T/PS CF	6 (16%)	3 (7%)
	5T/?	21 (48%)*	7 (17%)*
Worsening/recurrent symptoms		10%*	31%*
Diagnosis	CF (PS or PI)	18%*	62%*
	CFTR-RD	20%	23%
	none	64%*	15%*

n.s.: no significant differences between any of the groups. SD: standard deviation
Second mutations: significant difference of frequency of 5T/PI CF and 5T/?, (indicated with *, P = 0.008).
Diagnosis: significant difference of frequency of CF and not CFTR related symptoms, (indicated with *, P = 0.000).
Worsening/recurrent symptoms: Significant difference of frequency between the groups, (indicated with *, P = 0.018).

Table 6. 5T patients with a PI CF mutation in trans divided by number of TG repeats

Division according to TG repeats		**5T-TG11 N=4**	**5T-TG12 N=18**	**5T-TG13 N=4**
Mean age in years (range) n.s.		46 (18-66)	27 (3-69)	20 (1-36)
Gender (% male) n.s.		3 (75%)	14 (78%)	4 (100%)
Mean sweat chloride (mEq/l, SD) n.s.		37 (8)	49 (12)	55 (15)
Abnormal NPD n.s.		2 (50%)	13 (72%)	3 (75%)
Worsening/recurrent symptoms n.s.		1 (25%)	5 (28%)	1 (25%)
Diagnosis	CF n.s.	25%	59%	33%
	CFTR-RD n.s.	75%	18%	67%
	None n.s.	0%	24%	0%

n.s.: no significant differences between any of the groups.

Discussion

This study provides the largest cohort of 5T patients that has been examined systematically. It is an important illustration of the enormous variety in clinical consequences among patients with a 5T mutation, also in those who have the same mutation in trans. In addition, we demonstrate how NPD correlates with genotype, clinical outcome and diagnosis and how it can contribute to determining diagnosis and prognosis in these patients, supplementary to sweat testing.

Due to the retrospective setup of this study, it also faces relevant limitations; it is important to note that the length of follow up is variable between patients and for some patients has been quite short to accurately tell if symptoms have recurred, worsened of improved. Therefore diagnosis remained unclear in some patients.

Another limitation, as mentioned earlier, is that data on TG repeats is not available for all study subjects, especially as in Israel determination of TG repeats was not part of standard care. This explains why in 48 patients TG status is missing. As a result of this, the groups after division according to TG repeats are too small to achieve a significant conclusion about the difference between these groups. This limitation is important as TGm is earlier described as an important influence on splicing in 5T [9]. In addition, in the group of 28 patients in whom no second mutation was found (5T/?), there are 18 patients who only underwent panel testing of the most frequent mutations, but had no sequencing. This means that in those patients, a second mutation may have been missed, to that this group cannot be viewed as carriers.

This study reflects on patients with 5T who were referred for diagnostic evaluation, therefore the patients described are not a representation of the entire group of persons with a 5T allele; it is likely that there are many persons carrying a 5T allele, with or without a CFTR mutation in trans, who do not experience symptoms at all and thus are never referred to a CF center for evaluation. For our study, we confined our scope to only the subgroup of 5T patients in whom CF or CFTR-RD diagnosis is considered, as these can pose relevant dilemmas in CF diagnostic center. According to our results, NPD has shown to be useful in addition to the clinical picture to evaluate if the patient is at risk for worsening of symptoms over time, as it correlates better with outcomes compared to sweat test or genotype alone. There is very limited availability of earlier published research about NPD results in 5T patients. Kerem et al. evaluated sweat tests in 5T patients [7]. Segal et al. describe 2 patients with 5T (with W1282X and D1152H in trans) and recurrent pancreatitis who had borderline sweat tests and in whom the NPD results were also abnormal [23].

Noone et al. describe another 2 patients; one 5T homozygous, with borderline sweat test and one F508del/5T with abnormal sweat test. NPD was performed with methods

not comparable to our measurements, but is described as indicative for reduced CFTR function [24]. In another study by Sharer et al., only one of the included 5T patients (F508del/5T) underwent NPD. Calculated $e^{(\Delta amil/(\Delta Clfree+\Delta iso))}$ in this patient was normal at 0.58, sweat chloride was 34 mEq/l [25]. A few other studies involving 5T patients used NPD testing, however the 5T patients' results were not mentioned separately from the group they were included in [26,27].

These reports show that in patients with 5T mutations, genotype alone does not accurately define CF-diagnostic state. Similarly, in all four different genotype groups in our study, the clinical picture varies from CF to asymptomatic individuals.

The fact that the percentage of patients with an abnormal sweat test is distinctively higher in group 2, compared to group 1 and 3, stresses the importance of the effect of the mutation on the second allele to the CFTR residual function. There is no definite explanation for the high percentage of abnormal sweat test result in group 4, it is possible however that in this group based on genotype, an abnormal sweat test may have been the main reason to still refer the patient for further diagnostic evaluation.

In some cases, genotype does provide some prediction of symptom change over time: patients with a PS or varying clinical consequence second mutation all were stable or improved during follow up. In all 3 other genotype groups, clinical outcome could not be predicted based on genotype.

The finding that variation is large within groups with the same genotype should not surprise as this is also seen in patient groups with CF genotypes not involving 5T (for example F508del/F508del), caused by previously established factors such as modifier genes and environmental factors like infections in early life [6].

In the patient groups with known TG repeats, we found a correlation between lower TGm and lower sweat chloride as well as more frequent normal NPDs. This however was not statistically significant, as would be expected based on the limited group size. It is in line with the earlier findings that 5T-TG11 preserves more CFTR function than TG12 and TG13 [5,28,29].

However, patients with 5T-TG11 may develop CF as demonstrated in our study. This is in accordance with the findings of Magne et al. who described a patient homozygous for 5T-TG11 without any other CFTR mutation, who deteriorated around the age of 50 and recovered partially after starting ivacaftor [30]. This highlights the limitation of genotype and TGm state alone, as it is not always predictive of CF diagnosis or phenotype. Moreover, the fact that there are mutation-specific treatments for CF and drugs specifically for 5T may become available in the near future, underlines the importance of a better characterization of this group of patients.

Interestingly, sweat chloride did not correlate well with clinical outcomes in the studied group. A group of 17 5T patients was described by Kerem et al., of which 15 had sweat test results. A wide range of sweat test results was seen between the patients with no clear correlation with diagnosis, which is in line with our findings [7]. However, in the subgroup with a normal sweat chloride (<30 mEq/l) in our study, none of the patients were diagnosed with CF or CFTR-RD. This suggests that a sweat test is a valid diagnostic test to rule out CF in this particular group, taking into account the lower cut-off value to rule out CF.

Conclusion

There is much variation in clinical status among 5T patients. TGm state and NPD measurement help to define the clinical impact in these patients better than genotype and sweat test alone.

Although not fully discriminative between patients who will remain stable and patients who will experience worsening or recurrent symptoms, our data obtained from this group of 5T patients suggest that NPD measurement can help in estimating if a patient is at high risk for worsening of symptoms, and could thus aid the decision if the patient should attend a CF center.

All patients with a PS CF mutation in trans and all patients who have a combination of normal sweat test and normal NPD were stable or im- proving over time. Therefore we suggest that these patients may not require follow up in a CF center. In all other 5T patients who are referred for diagnostic workup, and do not have a 5T/PS CF genotype or a combination or normal sweat chloride and normal NPD, follow up should be considered by the physician after extensive diagnostic work up.

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CHAPTER 3

Correlation of CFTR modulator effects in Ussing chamber measurements with cryopreserved human nasal epithelial cells of people with cystic fibrosis to clinical treatment outcomes

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Submitted

ABSTRACT

Aim

The aim of this study is to validate the efficacy of CFTR modulator Ussing measurements in cryopreserved nasal brushing-derived airway epithelial cells (HNEC) of persons with cystic fibrosis (pwCF) and explore its correlation with clinical treatment effects.

Methods

Cryopreserved nasal brushing-derived human nasal epithelial cells (HNEC) were differentiated in air-liquid interface (ALI)-cultures and subsequently subjected to measurements in Ussing chambers. The effect of CFTR modulators was determined in ALI-HNEC cultures of 23 subject with CF. In vitro measurements were then correlated with clinical responses, including changes in sweat chloride concentration, FEV_1, and BMI of the participants.

Results

The response to forskolin/IBMX in CFTR modulator treated ALI-HNEC of CF subjects correlated with the change in sweat chloride concentration (SCC, Spearman R=-0.512, p=0.007) and FEV_1 (Spearman R=0.380, p=0.037), but not BMI (Spearman R=0.280, p=0.098).

Conclusion

CFTR modulator responses in Ussing chamber correlate with clinical effects (SCC and FEV_1 changes), providing proof-of-concept of utilizing Ussing chamber measurements in ALI-cultures with cryopreserved HNEC for predicting treatment effects in pwCF.

Introduction

The introduction of cystic fibrosis transmembrane conductance regulator (CFTR) modulating drugs (CFTR modulators) have brought an impactful change to cystic fibrosis (CF) therapy in recent years (1). However, the clinical effects of these drugs are highly variable among people with CF (pwCF), even for people with the same genotype (2). To address this challenge, it is proposed that individual responses to CFTR modulating drugs can be predicted through in vitro assays that quantify CFTR function, including electrophysiological Ussing chamber measurements in air-liquid interface (ALI)-differentiated human nasal epithelial cells (HNEC) of pwCF (3). In comparison to distal airway epithelial cells, HNEC can be easily sampled through minimally invasive nasal brushings, making them more suitable for evaluating drug efficacy at a personalized level. Furthermore, comparative studies between cultured distal airway epithelial cells and nasal airway epithelial cells have revealed similar effects of CFTR modulators in Ussing chamber measurements (4). These findings therefore suggest that HNEC can serve as a surrogate model for assessing CFTR function in the distal airways.

In previous studies, Ussing chamber measurements in ALI-HNEC of pwCF was determined to predict CFTR modulating drug efficacy in individuals with rare CFTR genotypes (5–7). Furthermore, in several cohort studies, significant correlations were established between in vitro drug responses measured in Ussing chambers and clinical outcomes, such as improvements in lung function and reductions in sweat Cl- concentration (8–11). These promising findings further emphasize the potential of applying Ussing chamber measurements in ALI-cultured HNEC as a predictive tool for assessing drug efficacy in pwCF. However, previous studies primarily relied on the use of freshly isolated HNEC. One limitation of this approach is the requirement for repeated samplings of nasal cells, for instance in cases where testing of novel CFTR drugs is needed. This issue can be overcome by using cryopreserved cells, which offer the advantage of enabling multiple usages without the need of repeated collecting of nasal brushings. However, the use of cryostored HNEC for Ussing chamber measurements have been relatively unexplored and require further investigation.

In our previous work, we have developed protocols for culturing nasal brushing-derived HNEC under chemically defined and feeder-free conditions (12,13). These protocols have proven effective in maintaining CFTR function in ALI-HNEC after cryostorage (12,14), offering the potential for utilizing cryopreserved cells in future experiments. In the present study, our objective was to further explore the correlation between the efficacy of CFTR modulators, as determined by Ussing chamber measurements in cryopreserved HNEC from pwCF, and the clinical effects of treatment.

Methods

Cell culture of nasal-brushing-derived epithelial cells

Nasal brushings of individuals with CF (n=23) and non-CF control subject were collected and stored with informed consent. This approach was approved by a specific ethical board for the use of biobanked materials TcBIO (Toetsingscommissie Biobanks), an institutional Medical Research Ethics Committee of the University Medical Center Utrecht (protocol ID: 16/586). HNEC were isolated from brushings and subsequently cryo-stored as previously described (12,13). For assessment of CFTR function and modulator responses, cryo-stored HNEC (passage 4) were first expanded in collagen IV (Sigma-Aldrich) coated wells, using a growth factor- and chemically-defined medium (Table 1). Afterwards, 0.2*106 cells were passaged on PureCol (Advanced BioMatrix) pre-coated 24 well transwell inserts (0.4 µm pore size polyester membrane, Corning) and differentiated as ALI cultures in ALI differentiation medium (Table 2). ALI-HNEC were differentiated for 18 days, medium was refreshed three times a week, and the apical surface of the cells was washed with PBS once a week. The trans-epithelial electrical resistance (TEER) was measured with an epithelial voltohmmeter (EVOM2, World Precision Instruments), to determine the airway epithelial barrier integrity of ALI-HNEC cultures during differentiation. The mean resistance was corrected for the surface area of the transwell insert (⌀ 6.5 mm) and depicted as $\Omega*cm^2$.

Quantitative real time PCR (qPCR)

RNA extraction, cDNA synthesis, and quantitative real time PCR was conducted in ALI-HNEC cultures as previously described (12). In brief, RNA was extracted using the RNeasy Mini Kit (Qiagen). Subsequently, cDNA was synthesized with the iScript cDNA synthesis kit (Bio-Rad), and qPCR was conducted with specific primer pairs (Table 3) using the iQ SYBR Green Supermix and CFX96 real-time detection machine (both Bio-Rad). Relative gene expression levels were normalized to housekeeping genes (ATP5B and RPL13A) using CFX Manager 3.1 software.

Immunofluorescence staining and imaging

Undifferentiated HNEC and ALI-differentiated HNEC cultures were stained and imaged as previously described (12), using primary antibodies listed in Table 4. Images were acquired with a Leica THUNDER imager (Figure 1b), and Leica SP8X confocal microscope (Figure 1f). Images were processed using LAS X software.

Ussing chamber measurements

Short-circuit current (Isc) measurements were conducted in ALI-HNEC cultures with an Ussing chamber system using a voltage clamp. Electrophysiological analyses were carried out along an apical-to-basolateral Cl^- gradient to maximize apical CFTR function measurements, by using a basolateral buffer consisting of 120 mM NaCl, 25 mM $NaHCO_3$, 3.33 mM KH_2PO_4, 0.83 mM K_2HPO_4, 1.2 mM $CaCl_2.H_2O$ and 1.2 mM $MgCl_2.6H_2O$, and

apical buffer consisting of 1.2 mM NaCl, 25 mM $NaHCO_3$, 3.33 mM KH_2PO_4, 0.83 mM K_2HPO_4, 1.2 mM $CaCl_2.H_2O$ and 1.2 mM $MgCl_2.6H_2O$ and 141 mM Na-gluconate (all Sigma-Aldrich). ALI-HNEC cultures from F508del/F508del donors were pretreated with VX-809 (10 μM), a combination of VX-661(10 μM)/VX-445 (5 μM) (all Selleck Chemicals), or vehicle (DMSO) for 48 hours. During measurement, ALI-HNEC were sequentially stimulated at the apical side with Benzamil (5 μM; Sigma-Aldrich) to block epithelial Na^+ channel-mediated currents, at the apical/basolateral side with VX-770 (5 μM; Selleck Chemicals), followed by a apical/basolateral stimulation with a mix of cAMP agonists forskolin (10 μM)/IBMX (100 μM) (both Sigma-Aldrich), and apical stimulation with the specific CFTR channel blocker CFTRInh-172 (5 μM; Sigma-Aldrich). Measurements were conducted at a bath temperature of 37°C and continuously gassing with 95% O_2/5% CO_2 to maintain a pH of 7.4. Tracings were recorded and analyzed with PowerLab and LabChart 6 (both AD Instruments).

Data analysis

Ussing chamber measurements in independent donors were conducted with two technical replicates for each experimental condition. Graphs were made with Prism 8 (GraphPad Software Inc.). Statistical analysis was performed using IBM SPSS 25.0 or Prism 8. To assess correlations, one-tailed Spearman R test was used, as this test is less susceptible to outliers in small groups compared to Pearson R.

Results

Patient characteristics

In total, 23 patients were included, 12 male and 11 female between the ages of 6-43. Genotypes are presented in Table 5. Clinical data at baseline and follow up are presented in Table 6.

Characterization of nasal-brushing-derived ALI-HNEC cultures.

Nasal-brushing-derived HNEC were isolated, cryo-stored, and differentiated in ALI-cultures (Figure 1a). Initially, HNEC were isolated in conventional 2D cell cultures using growth factor-defined and feeder layer-free conditions. The undifferentiated HNEC exhibited positive staining for integrin 6A, cytokeratin 5 (KRT5), and p63, indicating their phenotype as airway basal stem/progenitor cells (Figure 1b). Following isolation, HNEC were cryo-stored for subsequent usage. To perform Ussing chamber measurements, cryo-stored HNEC were expanded and then differentiated in ALI-cultures on transwell inserts. During ALI-differentiation, the formation of an epithelial barrier was observed, as indicated by significant increases in trans-epithelial electrical resistance (TEER) after 4 days, which persisted for 18 days of differentiation (Figure 1c). Differentiated ALI-HNEC exhibited significantly lower mRNA expression of airway basal cell markers and enhanced expression of secretory and ciliated cell markers compared to undifferentiated

cultures (Figure 1d). Furthermore, differentiated ALI-HNEC consisted of MUC5AC+ cells and β-tubulin IV+ cells, demonstrating a mucociliary phenotype.

CFTR function and modulator response measurements in ALI-HNEC cultures

Next, we investigated CFTR function and the effects of modulators in ALI-HNEC cultures by measuring Cl^- conductance measurements in Ussing chambers. These measurements were performed in ALI-HNEC cultures obtained from an healthy control (HC) subject, as well as from the 23 subjects with CF who were receiving treatment with CFTR modulators. After inhibiting ENaC with benzamil, we observed an increase in Cl^- conductance upon stimulation with Forskolin/IBMX in ALI-HNEC cultures from HC subjects (Figure 2a). This increase could be suppressed with chemical CFTR inhibitors. In contrast, in ALI-HNEC cultures from all subjects with CF, Cl^- conductance was largely attenuated (Figure 2b-d). Stimulation with VX-809/VX-770 (Figure 2e) or VX-661/VX-445/VX-770 (Figure 2g) resulted in increased Cl^- conductance, which could be suppressed with chemical CFTR inhibitors, indicating CFTR dependence. We also tested the effect of the CFTR potentiator VX-770 in ALI-HNEC cultures from CF subjects with F508del/S1251N genotype (Figure 2c) and individuals with F508del/G1249R and F508del/R117H (7T) genotypes (Figure 2f). Furthermore, we determined the effect of VX-661/VX-445/VX-770 in ALI-HNEC cultures from CF subjects with F508del/Y1092X (Figure 2d and g) and F508del/c.317+G>T genotypes (Figure 2g). On a group level, we observed a significant increase in Forskolin/IBMX-induced Cl^- conductance (ΔIsc FSK/IBMX), and a significant decline in responses to CFTR inhibitors (ΔIsc CFTRi), in all CFTR modulator treatment groups compared to vehicle and CFTR modulator-treated ALI-HNEC cultures from CF subjects (Figure 2e-g).

Correlation between CFTR modulator responses in CF ALI-HNEC and clinical responses

Finally, we calculated correlations (Spearman R) between the clinical responses after 6 months and the Ussing measurements (Figure 3). Correlations with sweat Cl- concentration were done for 22 subjects, due to lack of measurements in one subject. The efficacy of the CFTR modulators in Ussing chamber measurements were defined either as ΔIsc FSK/IBMX (Figure 3a-c) or (ΔIsc CFTRi) (Figure 3d-f). With ΔIsc FSK/IBMX, we found a significant correlation between Ussing results with sweat Cl^- concentration (R=-0.512, p=0.007) (Figure 3a). However, we did not observe a significant correlation between the ΔIsc CFTRi and sweat Cl^- concentration (R=0.2034, p=0.1819). In contrast, a significant correlation was observed between ΔIsc FSK/IBMX and ΔIsc CFTRi, with changes in FEV_1 (R=0.380, p=0.037, and R=0.5639, p=0.0025 respectively) (Figure 3b and d). In addition, we did not observe a correlation with BMI (R=0.280, p=0.098, and R=0.2865, p=0.0925 respectively) (Figure 3c and d).

Discussion

This exploratory study aimed to examine the correlation between CFTR modulator responses in Ussing chamber measurements, specifically using ALI-cultured cells derived from cryopreserved HNEC from pwCF, and the effects of treatment on clinical outcomes. Correlations were established between Ussing chamber measurements and real-life clinical effects, providing a practical and applicable tool for routine use, for patients whose mutations are not represented in a controlled clinical study. Our findings revealed significant correlations between CFTR modulator responses in Ussing chamber measurements and changes in sweat Cl^- concentration and FEV_1. These findings align with previous studies that employed freshly isolated HNEC for Ussing chamber measurements in ALI, suggesting that cryopreserved HNEC are also reliable for predicting CFTR modulator response measurements in pwCF.

Besides the usage of cryopreserved HNEC, our study deviates from previous research in several key aspects. Firstly, while previous studies propagated freshly isolated HNEC mainly using feeder cells (8–11), we followed previously established chemically and growth factor defined culture conditions for the propagation of HNEC (12,13). Indeed, Park et al. also examined CFTR modulator responses in HNEC expanded in feeder-free conditions and reported a correlation with changes in sweat chloride concentration (15). Our findings therefore provide further support for the suitability of feeder-free culture conditions for propagating HNEC used in CFTR modulator response measurements. Secondly, we also employed distinct culture conditions for ALI-cultures, which were serum-and bovine pituitary extract-free. This is crucial because previous comparative study has demonstrated varying effects of ALI-differentiation media on airway epithelial cell differentiation (16), which could result in different responses of CFTR modulators across studies. Furthermore, in contrast to several other studies, we incorporated a chloride gradient during the Ussing measurements. This deliberate inclusion enhances the magnitude of CFTR response to stimuli, as evidenced by previous research conducted by Bratcher and colleagues (17).

Our study furthermore had several limitations that should be acknowledged. Firstly, the number of CF subjects included in our study was limited, and they displayed significant differences in baseline characteristics. This variability makes it insufficient to accurately predict treatment effects based solely on Ussing chamber measurements for individual patients. The limited number of patients also makes it impossible to separately analyze the different mutation groups and identify if one group has stronger correlation between Ussing and clinical outcome compared to others. Visually, the correlation for the gating mutations seems specifically weak, however we cannot accurately verify that in this limited group. Additionally, our study focused on specific genotypes and a limited number of individuals who received treatment with the highly effective CFTR modulator triple therapy. It is furthermore notable that we only observed a correlation

between Ussing chamber measurement and sweat chloride concentrations when using ΔIsc FSK/IBMX values. Moreover, although we demonstrated the use of cryopreserved cells, we did not conduct a comparative study with freshly isolated cells. For this reason, we cannot draw conclusive differences in Ussing chamber measurements between cryopreserved and freshly isolated HNEC. Finally, in our study we used HNEC that had undergone 4 passages before ALI-culturing, whereas in other studies typically low passage cells were used. Previous work has shown a negative relation between passage number and CFTR function, but relations between CFTR function and passage number for the currently used culture technology still needs further studies (18,19). These factors may have influenced the outcomes observed in our study.

Altogether, we provide proof-of-concept of utilizing Ussing chamber measurements in ALI-cultures with cryopreserved HNEC for predicting treatment effects in pwCF. To gain further insight about the robustness and applicability of cryopreserved cells, further research is needed with larger sample sizes, diverse genotypes, highly effective CFTR modulators, comparative analysis between cryopreserved and freshly isolated cells, and careful consideration of passaging effects.

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Figure legends

Figure 1. Characterization of nasal brushing-derived HNEC.

a) Illustration showing the workflow of culturing nasal brushing-derived HNEC. b) Immunofluorescent staining of undifferentiated HNEC with DAPI (nuclei, blue), and the basal progenitor/stem cell markers integrin α6 (ITGA6, green), cytokeratin 5 (KRT5, cyan), and p63 (red). Scale bar equals 100 µm. c) The trans-epithelial electrical resistance (TEER) was measured in ALI-HNEC (n=3 independent donors) differentiated for 1, 4, 8, and 18 days. TEER values are shown as $\Omega*cm^2$ (means ± SD). d) mRNA expression was determined in undifferentiated HNEC (open boxes) and 18 days differentiated ALI-HNEC (grey boxes) (n=4-5 independent donors) of TP63, KRT5 (basal cell markers), SPDEF, AGR2, MUC5AC, SCGB1A1 (secretory cell markers), FOXJ1 and DNAI1 (ciliated cell markers). Results represent target mRNA expression normalized for the geometric mean expression of the housekeeping genes ATP5B and RPL13A, and are shown as boxplots with whiskers from minimum to maximum. e) Whole-mount immunofluorescent confocal imaging of ALI-HNEC differentiated for 18 days. Maximal projections of the apical side showing staining of MUC5AC (secretory cells) and β-tubulin IV (ciliated cells). Epithelial markers are shown in green, phalloidin in red was used as actin cytoskeleton staining. Scale bar equals 50 µm. Analysis of differences was conducted with a one-way ANOVA with Bonferroni post-hoc test (c), and paired t-test (d). * $p<0.05$, *** $p<0.001$.

Figure 2. Ussing chamber measurements in ALI-HNEC cultures of HC and CF subjects.

Representative short-circuit Ussing chamber tracings showing current measurements (Isc) in: a) HC, b) CF F508del/F508del, c) CF S1251N/F508del, and d) CF F508del/Y10992X ALI-HNEC cultures. For indicated donors, ALI-HNEC cultures were pre-treated with VX-809, VX-661/VX-445, or vehicle for 48 hours. During measurements, cells were treated sequentially with Benzamil, Forskolin (Fsk)/IBMX, VX-770, and CFTR inhibitor 172 (CFTRi). Quantification of changes in currents after Fsk/IBMX stimulation (Δ Isc Fsk/IBMX, upper panels) or after stimulation with CFTR inhibitors (Δ Isc CFTRi, lower panels) of Ussing chamber measurements was done in e) CF F508del/F508del stimulated with vehicle, VX-809/VX-770 (n=10 independent donors). f) Effect of vehicle or VX-770 was determined in of Ussing chamber measurements with ALI-HNEC of CF subjects with F508del/S1251N (n=7 independent donors), F508del/G1249R (n=1 donor), and F508del/R117H-7T (n=1 donor) genotypes. g) Furthermore, responses to VX-661/VX-445/VX-700 was determined in CF subjects with an F508del/F508del (n=2 independent donors), F508del/Y1092X (n=1 donor), or F508del/c.3717+5G>T (n=1 donor) genotype. Analysis of differences was conducted with a paired t-test (d). * $p<0.05$, ** $p<0.01$.

Figure 3. Correlation between Ussing measurements and clinical response.

Scatterplots showing correlation between Ussing and responses on clinical parameters after 6 months. Ussing response were calculated as difference in peak response in mA/

cm^2 to Forskolin/IBMX between vehicle and modulator treated cells (ΔIsc FSK/IBMX) (a-c), or difference in response in mA/cm^2 to CFTR inhibitors (ΔIsc CFTRi) (Figure d-f). Graphs depict Correlation between Ussing measurements and a,d) changes in sweat Cl^- concentration (SCC), b,e) changes in FEV_1, and c,f) changes in BMI. Spearman R test was used to determine correlations.

Figures

Figure 1. Characterization of nasal brushing-derived HNEC

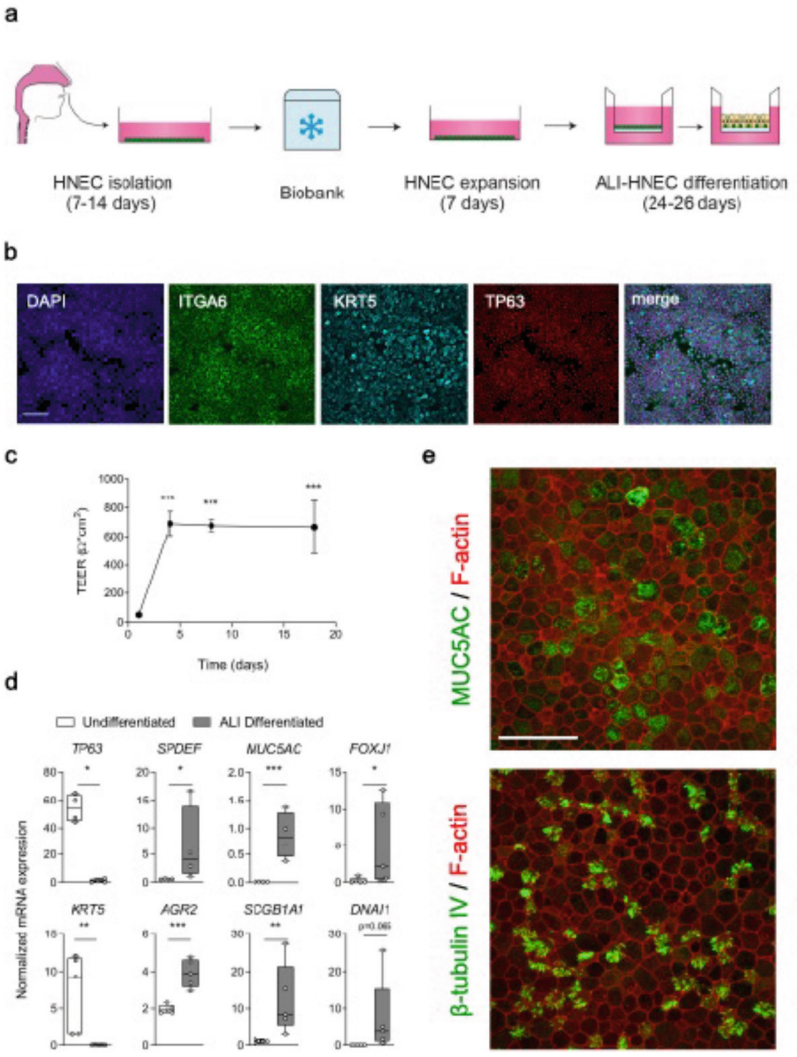


Figure 2. Ussing chamber measurements in ALI-HNEC cultures of HC and CF subjects

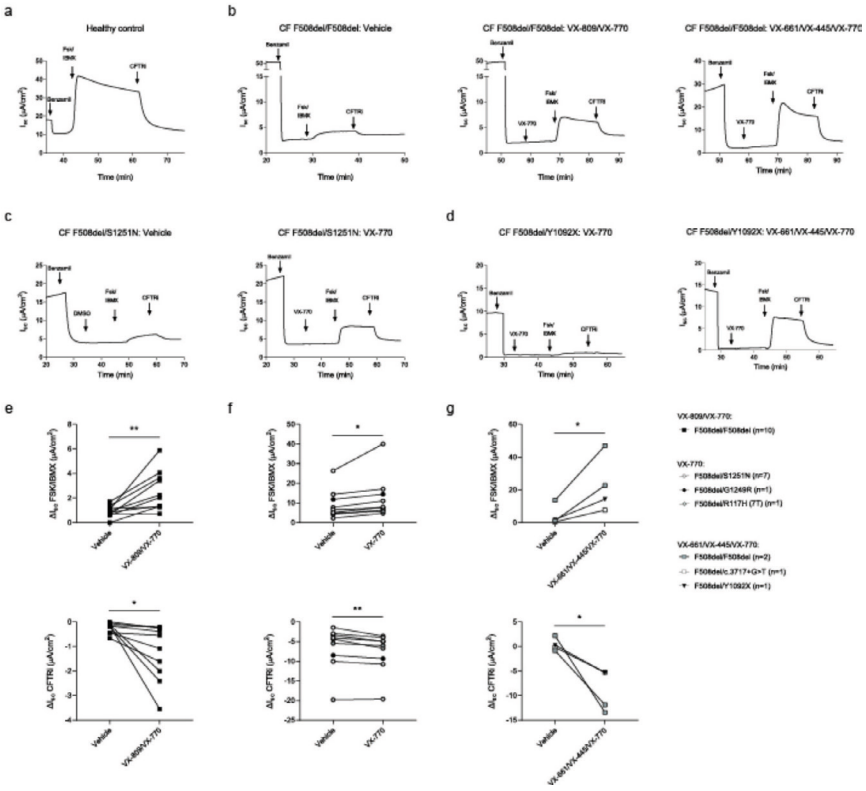
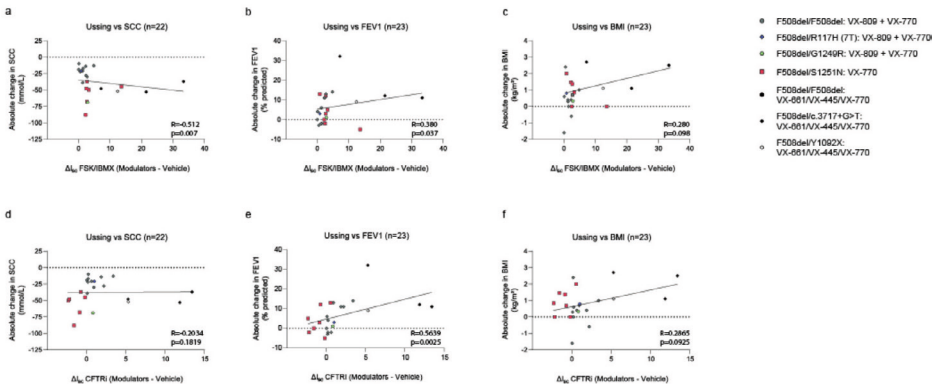


Figure 3. Correlation between Ussing measurements and clinical response



Tables

Table 1. Basal cell expansion medium

Reagent	Concentration
Bronchial epithelial cell medium-basal (BEpiCM-b)	49.6% % (v/v)
Advanced DMEM F12	44 % (v/v)
B-27 Supplement, serum free	2 % (v/v)
GlutaMAX Supplement	1 % (v/v)
HEPES (1 M)	10 mM
(±)-Epinephrine hydrochloride	0.5 µg/mL
Hydrocortisone	0.5 µg/mL
3,3',5-Triiodo-L-thyronine sodium salt	100 nM
N-Acetyl-L-cysteine	1.25 mM
Nicotinamide	5 mM
SB 202190 (p38i)	500 nM
DMH-1 (BMPi)	1 µM
A83-01 (TGF-βi)	1 µM
Y-27632 (ROCKi)	5 µM
DAPT	5 µg/mL
Recombinant human FGF-7	25 ng/mL
Recombinant human FGF-10	100 ng/mL
Recombinant human EGF	5 ng/mL
Recombinant human HGF	25 ng/mL
Rspondin 1 conditioned medium (from Rspo1 cells Cultrex®)	20 % (v/v)
Penicillin-Streptomycin	1 % (v/v)
Primocin	100 µg/mL

Table 2. Air-liquid interface (ALI)-differentiation medium

Reagent	Concentration
Advanced DMEM F12	98.5% (v/v)
(±)-Epinephrine hydrochloride	0.5 µg/mL
Hydrocortisone	0.5 µg/mL
3,3',5-Triiodo-L-thyronine sodium salt	100 nM
Penicillin-Streptomycin	1 % (v/v)
A83-01 (TGF-βi)	50 nM
TTNPB (Retinoic acid agonist)	100 nM
Recombinant human Epidermal growth factor	0.5 ng/mL
Recombinant Neuregulin-1β	0.5 nM

Table 3. qPCR primers

Gene	Forward primer (5'- 3')	Reverse primer (5'- 3')
ATP5B	TCACCCAGGCTGGTTCAGA	AGTGGCCAGGGTAGGCTGAT
RPL13A	AAGGTGGTGGTCGTACGCTGTG	CGGGAAGGGTTGGTGTTCATCC
TP63	CCACCTGGACGTATTCCACTG	TCGAATCAAATGACTAGGAGGGG
SPDEF	ATGAAAGAGCGGACTTCACCT	CTGGTCGAGGCACAGTAGTG
MUC5AC	ATTTTTTCCCCACTCCTGATG	AAGACAACCCACTCCCAACC
FOXJ1	GGAGGGGACGTAAATCCCTA	TTGGTCCCAGTAGTTCCAGC
KRT5	CCAAGGTTGATGCACTGATGG	TGTCAGACATGCGTCTGC
AGR2	GCAGGTGGGTGAGGAAAT	TTTGGCTCCAGGTTTGAC
SCGB1A1	CCAGACTCAGAGACGGAACC	TGAGGGTGACAGCGAGTTTC
DNAI1	tgcgaaactggacaaactgc	tgcgaaactggacaaactgc

Table 4. Primary antibodies

Antibody	Cat. No.	Origin	Isotype
ITGA6- AF 647 conjugated	**562494**	Rat	IgG2a
KRT5	ab17130	Mouse	IgG1
p63	ab124762	Rabbit	IgG
MUC5AC	ab198294	Rabbit	IgG
β-tubulin IV	MU178-UC	Mouse	IgG1

Table 5. Patient genotypes

Genotype	Number of patients
F508del/F508del	N= 12
F508del/S1251N	N= 7
F508del/G1249R	N=1
F508del/R117H	N=1
F508del/Y1092X	N=1
F508del/c.3717+5G>T	N=1

Table 6. Baseline and clinical follow up data: mean, (SD)

Modulating drug		FEV_1	Sweat chloride	BMI
Ivacaftor (N=9)	**Before start**	86.3 (25.5)	82.5 (19.6)	21.4 (5.5)
	Change after 6 months	+3.3 (6.0)	-53.3 (21.0)	+0.8 (0.7)
Lumacaftor/ivacaftor (N=10)	**Before start**	61.0 (23.1)	92.5 (9.9)	21.3 (2.1)
	Change after 6 months	+5.2 (6.7)	-21.3 (8.9)	+1.7 (1.0)
Elexacaftor/ tezacaftor/ivacaftor (N=4)	**Before start**	31.3 (2.6)	93.5 (10.9)	21.7 (2.7)
	Change after 6 months	+16.0 (10.7)	-47.5 (7.3)	+1.9 (0.9)

CHAPTER 4

Forskolin induced swelling (FIS) assay in intestinal organoids to guide eligibility for compassionate use treatment in a CF patient with a rare genotype

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ABSTRACT

Background

Forskolin-induced swelling of patient-derived organoids has been used to measure patient-specific CFTR function and CFTR modulator response. We present a case where CFTR function assessment in intestinal organoids was decisive for a patients' acceptance to a compassionate use program.

Case description

A 56 years old female with cystic fibrosis compound heterozygous for F508del and a rare CFTR allele (c.3717+5G>T) experienced rapid clinical deterioration. The forskolin-induced swelling assay on her rectal organoids was used to confirm that the rare mutation is a minimal residual function mutation, and that other CFTR modulators would not likely be effective. Based on these two criteria and her clinical status, she was accepted for compassionate use of elexacaftor/tezacaftor/ivacaftor and showed improvement in all clinical parameters.

Conclusion

This reports describes a first example that intestinal organoids were used to identify a previously unknown CFTR mutation as a minimal function mutation. The individual FIS-based definition of minimal residual function, response to ELE/TEZ/IVA and/or lack of response to other CFTR modulating drugs, may thus provide a tool for access to ELE/TEZ/IVA treatment for people with rare genotypes.

Introduction

Elexacaftor (ELE) in combination with tezacaftor (TEZ) and ivacaftor (IVA) is a next generation CFTR modulator treatment and has been approved for treatment of cystic fibrosis in October 2019 in the US and in August 2020 in the EU. ELE/TEZ/IVA is a triple CFTR modulating drug combination with higher efficacy compared to single and dual CFTR modulating drugs in subjects with the F508del/F508del genotype and for F508del compound heterozygous with defined minimal function CFTR mutations [1,2].

Many mutations associated with cystic fibrosis (CF) remain uncharacterized and have not been functionally annotated [3]. Patient-derived intestinal organoids facilitate the measurement of individual CFTR function through the forskolin-induced swelling (FIS) assay, enabling both the assessment of CFTR residual function and the effects of CFTR modulators. [4,5]. This assay measures CFTR-dependent ion and fluid transport into the organoid lumen that causes rapid organoid swelling. We report the case of an individual with CF who has a F508del compound heterozygous CFTR genotype and c.3717+5G>T who was originally rejected for compassionate of ELE/TEZ/IVA because her rare allele was not characterized and defined as a minimal function allele. Organoids were used for an individual assessment of CFTR function and classified her allele as minimal function, which guided inclusion into the ELE/TEZ/IVA compassionate use program.

Case description

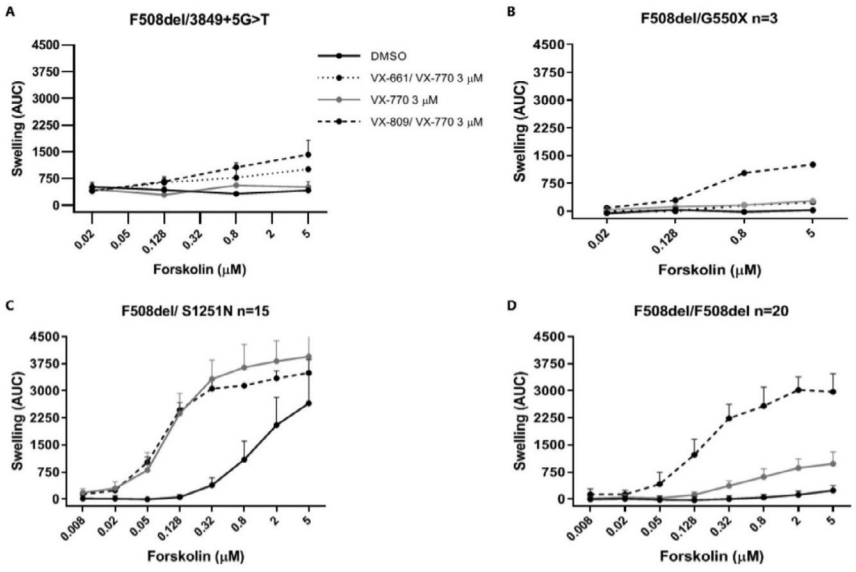
We present a 56 years old female with cystic fibrosis and a c.1521_1523del(F508del)/c.3717+5G>T genotype, who was diagnosed with CF in her first year of life. She has exocrine pancreatic insufficiency as well as CF related diabetes mellitus for which she uses insulin. She has had recurrent airway infections throughout her life, and is chronically infected with Pseudomonas aeruginosa.

In recent years, her clinical condition had deteriorated with frequent pulmonary exacerbations. In the previous year, she received multiple courses of antibiotics and 2 courses of prednisone because of pulmonary exacerbations, two of which required pro longed hospitalization for intravenous therapy. Her FEV_1 at this point was at 30% of predicted, with an annual average decline of 4% in the previous 3 years. Due to the severely impaired lung function and the frequent exacerbations, the disease had a grave impact on the patients' daily life. Because of the gradual deterioration in clinical condition, screening for lung transplantation was started, and concurrently her eligibility for ELE/TEZ/IVA treatment on a compassionate use basis was investigated. A request for inclusion into the compassionate use program was denied as the ultrarare c.3717+5G>T mutation was not designated as a minimal residual function mutation.

The individual agreed to provide rectal biopsies for generation of intestinal organoids and CFTR function measurement. An increasing dose of forskolin was used to test

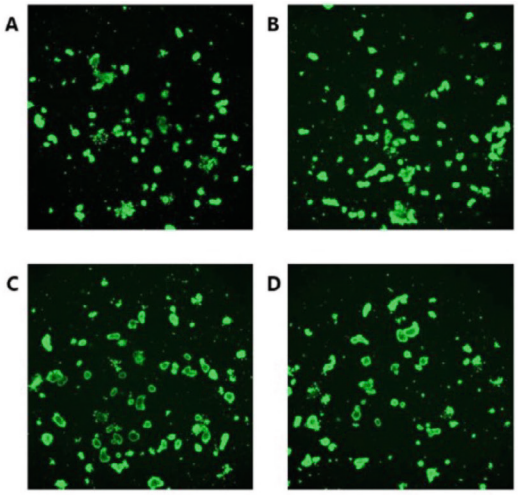
organoid swelling, and showed no baseline swelling of her organoids indicative of a minimal residual function genotype and only no (ivacaftor) or limited swelling after lumacaftor/IVA or TEZ/IVA incubation, quantitatively comparable to average responses of organoids with one F508del allele combined with a nonsense mutation (G542X) (Fig. 1, 2). A combination of ELE/TEZ/IVA was not tested on the organoids, as elexacaftor was not yet available for in vitro testing in our laboratory. When it became available, we encountered an inability to restore living cultures from the patients' biobanked organoids to perform a new FIS assay. We deemed it unethical to request new biopsies from our patient if it would solely be for this purpose.

Figure 1. Graphs of the FIS assay with responses to ivacaftor (VX-770), ivacaftor/lumacaftor (VX-770/VX-809), ivacaftor/tazecaftor (VX-770/VX-661)



A: Our case patients' organoids (two measurements)
B: Reference organoids (N=3) derived from patients with F508del/G550X genotype
C: Reference organoids (N=15) derived from patients with F508del/S1251N genotype
D: Reference organoids (N=20) derived from patients with F508del/F508del genotype

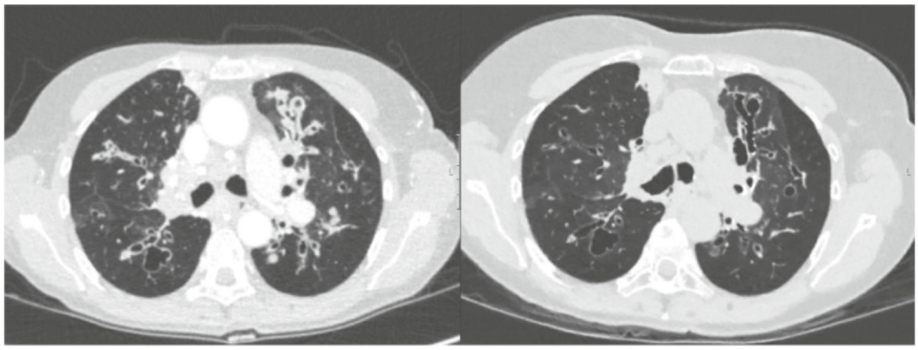
Figure 2. Images of FIS assay on rectal organoids of our case patient



A: Before stimulation with forskolin
B: DMSO control after maximum forskolin stimulation (5.0µM)
C: VX-809/VX-770 pretreated, after maximum forskolin stimulation (5.0µM)
D: VX-662/VX-770 pretreated, after maximum forskolin stimulation (5.0µM)

Based on these results in combination with her clinical status, this patient was allowed to join the compassionate use program and start ELE/TEZ/IVA treatment. After only days of treatment, she started coughing less and feeling more energetic. After 6 months of treatment, her FEV_1 had increased to 52% of predicted (+22%), her sweat chloride had dropped to 49 mmol/l (−48 mmol/l) and her chest CT (Fig. 3) showed markedly less mucus plugging, bronchial wall thickening and peribronchial thickening compared to a CT obtained 2 months before the start. Her CFQ-R scores improved not only on the respiratory domain (44 to 83 on the 100 point scale), but also on the subdomains health, vitality, social, physical, role and digestive. In the first 12 months of treatment she had no pulmonary exacerbations. After 18 months of treatment, her FEV_1 had improved further to 57% of predicted, the highest level since at least 16 years.

Figure 3. CT scan images of our patient one month before start (left) and three months after start of treatment (right)



Discussion

We report the first case, to our knowledge, in which organoid FIS was used to argue eligibility for triple CFTR modulating treatment on a compassionate use basis. One condition for applicability to this case, is that the technique should be able to verify if an ultra-rare mutation of which the consequences are unknown, is a minimal function mutation.

The FIS assay provides a CFTR dependent readout for individuals that correlates with other biomarkers of CFTR function, clinical disease severity and CFTR modulator responses [4,6,7] Baseline residual function measurement was clearly absent and different from organoids with residual function alleles (Fig. 1) [4,6]

Strong responses to the then available first generation modulators were also absent. By quantitatively comparing the current measurements to published average organoid responses with defined CFTR genotypes [5], the c.3717+5G>T allele would be designated as a non-functional class I splicing mutation. This report shows that individual CFTR function measurements can be used to classify CFTR genotypes as minimal function genotypes. This is different from the currently used CFTR allele-specific typing by Fisher Rat Thyroid cells that quantifies CFTR function upon introducing mutations in a defined genetic context. This system does not integrate individual genetic variability within the CFTR gene and other modifiers that could impact on individual function, and is also not equipped to assess splice mutations in a direct and individual manner as was studied here. We anticipate that the methodology used here that is based on individual CFTR function assessment in organoids could help more people with CF and rare, uncharacterized mutation to get access to critical treatment.

An issue that should be addressed in the future, is the current lack of a cut-off value for the definition of minimal function based on the FIS assay or any other measure. In our patients' case it was very clear that residual function was close to zero in the grown organoids. However, the thresholds that discriminate between individuals having minimal residual function or 'more-than-minimal' remain unclear for organoid FIS or any other individual CFTR function test or clinical feature. This unclarity hampers the implementation of the FIS test or other criteria (e.g. based on pancreatic insufficiency or sweat chloride concentration) in facilitating access to ele-tez-iva for individuals with uncharacterized CFTR genotypes.

Conclusion

We show a first application of the FIS assay on patient-derived organoids to define minimal CFTR residual function of genotypes and associated alleles in the context of eligibility for compassionate use of ELE/TEZ/IVA. This individual-based approach may provide a timely solution for assessment of drug eligibility for people with CF who have rare genotypes.

Declaration of competing interest

HGM Heijerman has provided assistance to Vertex pharmaceuticals, the manufacturer of lumacaftor/ivacaftor, as member of the advisory board, speaker and investigator in clinical studies.; BL Aalbers has assisted in clinical trials for Vertex as a sub-investigator.; CK van der Ent reports grants from Vertex, outside the submitted work.; JM Beekman and CK van der Ent are inventors on patent(s) related to organoid swelling, and received royalties from 2017 onwards.

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Part II
Clinical follow-up parameters after start of CFTR modulating treatment

CHAPTER 5

Clinical effect of lumacaftor/ivacaftor in F508del homozygous CF patients with FEV_1 ≥ 90% predicted at baseline

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ABSTRACT

Objective

The first available CFTR modulator combination for homozygous F508del patients, lumacaftor/ ivacaftor, has not been tested in patients with percentage predicted (pp) FEV_1 > 90 in the phase III trials. The objective of this study is to share real life experience about treatment results in this group.

Methods

In this retrospective observational study, patients aged 6 years or older starting on lumacaftor/ivacaftor in standard care were in strict follow up. For these patients, data were obtained about FEV_1, BMI, CFQ-R and sweat chloride before start and after 6 months of treatment, and data about FEV_1 and BMI were recorded every 3 months. Exacerbations were recorded continuously.

Results

We identified 40 patients with a $ppFEV_1$ ≥90 at the start of lumacaftor/ivacaftor who had been in follow up for at least 12 months. After 12 months, $ppFEV_1$ was unchanged, whereas mean absolute change in BMI was + 0.88 (p = 0.001) with a mean change in SDS for BMI of + 0.26 (p=0.014). Mean CFQ-R overall score at 6 months improved by 2.6% (p = 0.004) and mean decrease in sweat chloride was -27.3 mEq/L (p = 0.000). Exacerbation rate declined from 1.03 to 0.53/person/year (p = 0.003). One patient discontinued treatment in the first 12 months because of progression of CFRLD, two paused treatment but resumed later.

Conclusion

Homozygous F508del patients starting lumacaftor/ivacaftor at $ppFEV_1$≥90 improved significantly in nutritional status, sweat chloride levels and exacerbation rate, but did not respond in $ppFEV_1$. Treatment is well tolerated in this patient group. These effects make it worth considering to treat this group of patients with lumacaftor/ivacaftor.

Introduction

F508del is the most common CFTR mutation, present on 66.7% of alleles in Cystic Fibrosis (CF) patients in Europe, and even more frequently in Dutch patients. [1] It affects folding, trafficking, and function of the CFTR protein usually leading to a severe CF phenotype. [2] Targeted therapy for this mutated protein consists of a combination of a CFTR corrector, enhancing processing and trafficking and a potentiator, increasing the open probability of the chloride channel. [3].

The first available combination therapy for treatment of F508del homozygous patients regardless of $ppFEV_1$ is lumacaftor/ivacaftor (Orkambi, Vertex pharmaceuticals). In the phase III randomized clinical trials for this drug, patients with an FEV_1 between 40 and 90% predicted were included. A modest improvement in FEV_1 and BMI and a significant decrease in pulmonary exacerbations was observed in comparison to placebo. [4,5]. Since the introduction of lumacaftor/ivacaftor as part of usual care for F508del homozygous patients, data have become available regarding the clinical effect in patients who started this treatment with an FEV_1 below 40% predicted. In this group, it proved to be beneficial with a gain in FEV_1 after 3 months, while BMI remained unchanged. [6] However, the clinical effect of lumacaftor/ivacaftor treatment in patients with a baseline FEV_1 greater than or equal to 90% predicted has not yet been shared, despite the drug being available in this population. The aim of this study is to evaluate real life effects of lumacaftor/ivacaftor treatment in this specific patient group.

Methods

F508del homozygous CF patients from the UMC Utrecht CF center starting treatment with lumacaftor/ivacaftor were followed according to a pre-determined standardized protocol. We retrospectively collected this data for those patients whose $ppFEV_1$ was ≥90 at the time of starting treatment. To make sure no patients were missed, every CF patient was screened for the inclusion criteria before starting treatment with lumacaftor/ivacaftor. All patients were 6 years or older, as lumacaftor/ivacaftor was not available for children under 6 years of age during our study period. Data was collected between November 2017 and June 2019.

Before the start of the treatment, patients visited the outpatient clinic for a consult with their physician and baseline measurements: sweat test, spirometry, weight and length, CFQ-R. At the after 3-month visit, spirometry and measurement of height and weight were repeated. BMI was calculated from weight and height, which were measured in the outpatient clinic before spirometry. As the majority of the patients described are children, SDS (standard deviation score, also known as Z-score) for BMI was also calculated for all patients up to 20 years old (as SDS for BMI is only validated

for age under 21). For this calculation, reference data collected by TNO (the Netherlands Organization for applied scientific research) were used, from an extensive pediatric cohort in 2010. [7] All of the measurements performed at baseline were repeated at the after 6 months visit. Only spirometry and measurement of height and weight were repeated at the after 9 and 12-month visits.

Sweat chloride concentration measurement was performed according to the standard operating procedure of the European Cystic Fibrosis Society Clinical Trials Network (ECFS-CTN). [8] Macroduct Sweat Collection System (Wescor) was used for collection of the sweat. For analysis of the sweat chloride concentration argentometry was used. Spirometry was carried out according to the current European Respiratory Society (ERS) guidelines using reference data from the Global Lung Function Initiative (GLI) to calculate the $ppFEV_1$ [9,10].

Exacerbation data was collected continuously. Exacerbations were defined as either an oral or intravenous course of antibiotics started because of worsening of respiratory symptoms, and frequency of exacerbations was recorded accordingly for the year before and after start of treatment.

CFQ-R score was reported in percentage of maximum score, because different versions of the tool were used; the teen and adult version for patients 14 years or older (maximum score 192), the version for older children for ages between 11 and 14 (maximum score 140) and parent version for children ≤10 years (maximum score 176).

The data was analyzed using IBM SPSS Statistics 23 analysis software. Mean value and standard deviations (SD) were calculated for all baseline values. Paired T-test was used to compare the measured data at baseline to those at the subsequent follow up visits. To determine statistical significance, the cut-off for p-value was set at <0.05.

Results

Forty patients with CF met inclusion criteria. 14 of them were 18 years or older, 13 patients were 12 to 18 years old and 12 patients were 11 years or younger. All had been on lumacaftor/ivacaftor for at least 12 months with regular follow- up as described above. Baseline characteristics are described in Table 1. Only one patient with $ppFEV_1 > 90$ had not started treatment despite eligibility, because of his good clinical condition and parental concerns about possible side effects on the long term.

Table 1. Mean baseline values per parameter. Before lumacaftor/ivacaftor treatment *N* = 40

Sex	**18 female, 22 male**
Age (SD) years, at start	15.3 (6.2)
$ppFEV_1$ (SD)%	98.63 (7.71)
Change in $ppFEV_1$ in the year before start (SD)	−3.32 (6.22)
BMI (SD) kg/m^2 [a]	21.64 (2.63)
SDS for BMI[b]	0.234
CFQ (SD)% of maximum	87.1 (8.2)
Sweat chloride (SD) mEq/l	96.14 (11.45)
Number of exacerbations in the months before start	24 (0.60/person/6mo) 32 (0.80/person/9mo) 41 (1.03/person/12mo)

[a] *N* = 14, BMI was only reported for patients ≥ 18 years.
[b] *N* = 26, SDS for BMI was only reported for patients < 18 years.

In daily practice, not every follow up visit was exactly at the stated time point of 3, 6, 9 or 12 months after start. Maximum deviation from the exact time point was +/− 9 days for the after 3 months visit, +/−15 days after 6 months, +/− 23 days after 9 months and +/− 34 days at one year.

After 3 months (Table 2), $ppFEV_1$ was stable; mean change was +0.24% (p = 0.824). Mean change in BMI was not significant, with a mean change of +0.38 kg/m^2 (p = 0.052), and mean change of SDS for BMI of −0.025 (p = 0.761). After 6 months, $ppFEV_1$ was still unchanged (mean change +0.15%, p = 0.882), whereas mean change in BMI was +0.39 kg/m2 (p = 0.016), with a mean change in SDS for BMI of +0.147 (p = 0.090). After 9 months, $ppFEV_1$ remained constant (−0.43%, p = 0.710), while mean BMI continued to rise with a change of +0.60 (p = 0.019) from baseline. SDS for BMI in the patients under 18 years old also increased, with a mean change of 0.232(p = 0.023). At the visit after one year, the effect on BMI (+0.88, p = 0.001) and SDS BMI (+0.264, p = 0.014) increased, while $ppFEV_1$ still remained constant (−0.10, p = 0.431). When comparing the change in $ppFEV_1$ in the year after start to the change in the year preceding lumacaftor/ivacaftor treatment, there is a change in the lung function trend of these patients: mean decline of $ppFEV_1$ in the previous year had been 3.32 (change in decline of $ppFEV_1$ was 2.24, p = 0.159).

Table 2. Mean values after 3, 6, 9 and 12 months of lumacaftor/ivacaftor treatment

	3 months after start N = 40[a]	**6 months after start N = 40[b]**	**9 months after start N = 40[c]**	**12 months after start N = 40[d]**
$ppFEV_1$ (SD) % Change from baseline *p*-value	98.86 (8.56) 0.24 $p = 0.824$	98.77 (9.33) 0.15 $p = 0.882$	98.26 (10.31) −0.42 $p = 0.710$	98.7 (10.55) −0.10 $p = 0.431$
BMI (SD) kg/m^2 Change from baseline *p*-value	21.70 (2.54) 0.38 $p = 0.052$	22.04 (2.63) 0.39 $p = 0.016$	22.45 (2.77) 0.60 $p = 0.019$	22.55 (2.80) 0.88 $p = 0.001$
SDS for BMI Change from baseline *p*-value	0.245 −0.025 $p = 0.761$	0.380 0.147 $p = 0.090$	0.496 0.232 $p = 0.023$	0.523 0.264 $p = 0.014$
CFQ (SD)% of maximum Change from baseline *p*-value		89.9 (6.4) 2.8 $p = 0.004$		
Sweat chloride concentration (SD) mmol/l Change from baseline *p*-value		67.65 (15.12) 28.49 $p = 0.000$		
Number of exacerbations Change compared to same period before start *p*-value		14 (0.33/person) −10 (−0.27/person) $p = 0.039$	18 (0.45/person) −14 (−0.35/person) $p = 0.025$	21 (0.53/person) −20 (−0.50/person) $p = 0.003$

a $N = 40$ for all parameters except SDS BMI, only <18 years ($N = 26$) and BMI, only ≥18 years ($N = 14$).
b $N = 40$ for all parameters except CFQ ($N = 29$), sweat test ($N = 37$), SDS BMI ($N = 26$).
c $N = 40$ for all parameters except $ppFEV_1$ and BMI (missing: 2) SDS BMI ($N = 26$).
d $N = 40$ for all parameters except $ppFEV_1$ and BMI (missing: 2) SDS BMI ($N = 26$).

Although the mean CFQ-R overall score improved by 2.8% (p = 0.004), the domain where improvement was noticed was highly variable among patients. Of the 29 patients who completed the CFQ-R before starting treatment and after 6 months, 22 patients had improved in CFQ-R score. In 6 of them, the score improved more than 5%. The other 7 patients had stable or deteriorated scores, none of them had a score more than 5% lower. Sweat chloride change was also significant with a mean difference of −27.3 mmol/L (p = 0.000). In our group, the patients under 18 years had a more pronounced response in sweat chloride (mean difference of 31.3 mmol/l) compared to the patients 18 and over (mean response 24.5 mol/l).

In the 6 months preceding lumacaftor/ivacaftor treatment, a total of 24 exacerbations occurred (0.6 per person in 6 months). In the 6 months after the start, this had decreased to 14 exacerbations (0.33 per person in 6 months, p = 0.039). When 9 months before and after start were compared, the mean number of exacerbations was reduced from 0.80 exacerbations per person to 0.45/person. In the 12 months after start, mean exacerbation rate had changed to 0.53/person compared to 1.03/person in the year before. In the year preceding treatment, all exacerbations were treated orally in the home setting. In the year after start, one exacerbation required IV treatment.

Lumacaftor/ivacaftor treatment was stopped permanently in one patient, due to progressive CF related liver disease. This patient was excluded for analysis because discontinuation occurred after only two months of treatment and the patient thus did not receive therapy until one of the follow up visits. In two other patients treatment was paused due to gastro-intestinal symptoms, but restarted later without problems. As discontinuation in both patients was brief and did not involve the time points of 3 and 6 months follow up, they were not excluded for analysis.

Discussion

This study provides information about real life treatment effects of lumacaftor/ivacaftor in F508del homozygous patients for a subgroup with well-preserved lung function, that has not been previously evaluated.

Theoretically, this should be the group that benefits most of corrector-potentiator combination therapy in the long term, as improvement of CFTR function in an early stage of disease may prevent structural damage to the lungs and preserve lung function. Such an effect will take many years of treatment to evaluate. On a relatively short term however, we found distinct results of therapy. The increase in BMI and CFQ in combination with the reduction in exacerbation rate are clinically relevant in this group with a lung function that is not causing limitations.

Lung function decline is typical in CF, with a rate of ppFEV$_1$ decline of about 1% per year in pancreatic insufficient patients. [11] This indicates that a ppFEV$_1$ change of 0% could be a relatively favorable outcome. It is however somewhat bold to state this for the specific group that is discussed here, as these patients have a well preserved lung function, which means that they do not follow the described pattern of average lung function decline in CF patients.

In the F508del homozygous patients with lower baseline ppFEV$_1$, lumacaftor/ivacaftor treatment effect is known from earlier research: In the phase III trials for the drug (TRAFFIC and TRANSPORT, Wainwright et al.), patients with FEV$_1$ 40–90% predicted were included. After 24 weeks of follow up, mean change in ppFEV$_1$ was 2.6–4.0%. [4].

Clinical effect of treatment in a patient selection with baseline ppFEV$_1$ below 40% predicted was described by Hubert et al., who described that after 1 and 3 months of treatment, there was a significant gain in ppFEV$_1$. There was no effect on other parameters such as BMI and CFQ-R in these patients. [6].

We can conclude that our findings of treatment effect in the group with well-preserved lung function, contrast with those in the group with severely affected lung function, mainly on BMI. The follow up period may be a factor in this; in our study we see BMI gain increase over time, but with this effect only starting to show and not yet significant at 3 months of treatment, which is the end of follow up in the study by Hubert et al.

Importantly, in our study group exacerbation rate in the year following start of lumacaftor/ivacaftor was half of the rate compared to the year before start. In the phase III trials reduction in exacerbation frequency was 30–39% compared to placebo, which is comparable to our findings, although this real-life observational study did not compare treatment to placebo. Hubert et al. did not discuss exacerbation frequency due to the short follow up time. From the group of patients we identified, only one patient discontinued treatment for more than 4 weeks. This is distinctively less than described by Wainwright et al. (4.2% discontinued) and Hubert et al. (30% discontinued). This is not surprising as the patients with more severe CF lung disease will be more prone to respiratory adverse events and these were the main reason for discontinuation in those studies.

A remarkable finding in this study is the large mean response in sweat chloride concentration on therapy, which stands out from the much smaller effects described in other studies of the effect of lumacaftor/ivacaftor on sweat chloride in F508del homozygous patients, such as the phase II placebo-controlled trial for the drug by Boyle et al. In that study, a mean reduction in sweat chloride of 10.3 mmol/l was reported for the group that was treated with 400/250 mg twice daily, which is now used as standard dosage. [12] We suspect that one of the contributing factors could be the relatively

young age of the patients in our study; 65% of the patients were younger than 18 at the time of starting lumacaftor/ivacaftor, whereas the phase II study only included patients 18 years or older. Within our study group we see that the adolescents and children have a larger reduction in sweat chloride compared to the adults, which leads us towards this hypothesis, along with reports sweat test changes on ivacaftor, showing larger reduction of sweat chloride in younger children. [13,14].

Our study faced several limitations. Firstly, comparison of our results to those of the lumacaftor/ivacaftor phase III trials is limited by the fact that those were performed as randomized placebo controlled trials, whereas in our study the treatment effects were compared to the values from the same patients before the start of therapy. Our study in itself can therefore not rule out a placebo effect, however this was not a strong effect in the mentioned randomized controlled trials for lumacaftor/ivacaftor. Secondly, missing data occurred as patients occasionally missed an outpatient clinic visit. It is uncertain if this introduces any bias in the data, but it is likely that a patient who is doing particularly well is more inclined to skip a visit to the hospital compared to a patient experiencing lots of symptoms and/or adverse events. It is highly unlikely for the Dutch CF population, that a patient sought attendance for CF-related problems outside the CF clinic, without involving their own physician.

Due to the pairing for T test, the baseline value used to indicate 'change from baseline' in Table 2, may differ slightly from the value in Table 1 if any follow up data were missing.

Missing data were particularly prevalent in the CFQ-R. A possible main factor in this is that it is not a standard part of the outpatient clinic visits and thus could be more easily forgotten by both the patient and the caregiver. Additionally, when presenting the CFQ-R results we deliberately chose to present the percentage of the total score, instead of the amount of points for one specific domain. This is because we were interested in the overall effect of patient perceived CF related health, and not specifically in the respiratory symptoms, which were usually not the main issue in this specific group. We observed that improvement in CFQ-R score in this study was diffuse and did not involve one specific domain, endorsing the choice to present the percentage of overall CFQ- R score. However, all of the before mentioned studies describing lumacaftor/ivacaftor treatment effects present CFQ-R scores for the respiratory domain only, making it impossible to compare those to the outcomes in this study. A recent study on validation of an electronic version of CFQ-R, by Solé et al., reported marginal changes in electronic CFQ-R after 15 days on every separate domain in a group of clinically stable CF patients. They do not state anything about changes in total score and the study did not investigate the minimally clinically important difference, so that it remains challenging to put our findings on total score into perspective. [15].

Treatment discontinuation rate was exceptionally low in our study. We consider the favorable respiratory status of the patients as a main reason for this; if we compare discontinuation rate in the phase III trials for lumacaftor/ivacaftor to the description of effects of the drug in patients with $ppFEV_1$ <40% [4,6], we see a marked difference where patients with a lower $ppFEV_1$ are more likely to discontinue due to respiratory side-effects, which have more impact on patients with a poor lung function. Patients in the present study, with a very well preserved lung function, can be expected to be less prone to discontinuation due to respiratory events.

Considering the fact that lumacaftor/ivacaftor is a costly therapy, and the patient group we included in this study did not require many hospitalizations for IV treatment of exacerbations, the treatment will not reduce, but rather increase short term healthcare expenses in this group of patients. This study has not investigated if the improvement in a patients clinical status leads to more participation in study and/or work, which would also influence considerations about cost-effectiveness on the long term.

Conclusion

Homozygous F508del patients with well-preserved lung function starting lumacaftor/ivacaftor gain in nutritional status and quality of life, and also respond well in sweat chloride levels, but they do not respond in $ppFEV_1$ in the first year of treatment. Treatment is well tolerated. Although data on treatment outcome on a longer term are not yet available, we conclude that the improvement in BMI and quality of life, accompanied by a decline in exacerbation rate, make it worth considering to treat this group of patients with lumacaftor/ivacaftor.

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CHAPTER 6

Females with cystic fibrosis have a larger decrease in sweat chloride in response to lumacaftor/ivacaftor compared to males

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ABSTRACT

Aim

To explore which patient-related factors influence sweat test response to CFTR modulators, as well as examining the correlation between the sweat chloride response and $ppFEV_1$ or BMI response, using systematically collected real-life clinical data.

Methods

160 CF patients were identified who had used lumacaftor/ivacaftor for at least six months. Of these patients, age, sweat chloride levels, $ppFEV_1$ weight and BMI at the start of treatment and after 6 months were collected retrospectively. Pearson and Spearman tests were performed to assess correlations.

Results

Females compared to males in this group showed a larger response in sweat chloride (mean difference 10.6 mmol/l, 95% CI: 5.7–15.4) and BMI (mean difference 0.27 kg/m^2, 95% CI: 0.01–0.54). A modest but significant correlation was found between patient weight and sweat chloride response (Pearson R = 0.244, p = 0.001), which diminished upon correction for the other factors. The correlation between sex and sweat chloride response remained; R = 0.253, p = 0.001. Sweat chloride response did not correlate with $ppFEV_1$ change or BMI change at 6 months after start of therapy.

Conclusion

Sweat chloride response is larger in females compared to males, which also explains the negative correlation of weight with the response in sweat chloride concentration after start of lumacaftor/ivacaftor. Sweat chloride response does not correlate with the responses in $ppFEV_1$ and BMI. This information may help the interpretation of sweat test results acquired for the follow up and evaluation of CFTR modulating treatments, and warrants further investigation into the underlying mechanisms of sex differences in response to CFTR modulators.

Introduction

As it has been long known that the sweat gland is affected in Cystic Fibrosis (CF), the chloride concentration in induced sweat has been the gold standard in CF diagnostics for decades [1].

Recent advances in CF treatment include the development of CFTR modulating molecules, directly influencing the proteins' transport to the cell membrane and the open probability of the ion channel. In the follow up of patients who start with these CFTR modulating drugs, the sweat test is increasingly used as an in vivo outcome measure for CFTR function restoration [2,3].

In literature, one can recognize a pattern of greater sweat chloride responses to ivacaftor in very young children with CF [3], compared to older children and adults, suggesting a correlation between age and magnitude of sweat chloride response [4,5].

It is important to verify this possible effect of age on sweat chloride response and to assess if other factors influence this response, as the sweat test is commonly used as a monitoring test after commencing CFTR-targeted treatment in a patient. However little is known about the influence of factors such as age, weight and sex on the magnitude of the sweat chloride response, and about the correlation between sweat chloride response and other outcome measures such as lung function or BMI, which have direct relevance to the patient.

This study aims to explore which patient related factors are correlated to sweat chloride response to treatment with lumacaftor/ivacaftor, and if this outcome measurement correlates with clinical outcome parameters.

Methods

All homozygous F508del CF patients treated in the UMC Utrecht CF center were included who had used lumacaftor/ivacaftor for at least six months. All of them had already consented to the use of their clinical data for scientific research. All of these patients were 6 years or older, as for children under 6, lumacaftor/ivacaftor was not yet available at that time.

Before the start of lumacaftor/ivacaftor treatment, patients had visited the outpatient clinic for a consult with their physician and baseline measurements, including sweat test, spirometry, weight and length (for BMI). After 3 months, spirometry was repeated along with measurement of height and weight. All of the measurements performed at baseline were repeated at the visit after 6 months. Patients who had used lumacaftor/

ivacaftor for a shorter period of time (N = 6) or in whom baseline and/or follow up sweat test was missing for any reason (N = 7), were excluded. No patients had missing data on $ppFEV_1$ or BMI at baseline or in follow up.

Sweat for chloride concentration measurement was collected using Macroduct Sweat Collection System (EliTechGroup), and performed according to the standard operating procedure of the Euro- pean Cystic Fibrosis Society Clinical Trials Network (ECFS-CTN) [6]. For analysis of the sweat chloride level argentometry was used. Spirometry was carried out according to current European Respiratory Society (ERS) guidelines and reference data by the Global Lung Function Initiative (GLI) was used to calculated percentage predicted of FEV_1 ($ppFEV_1$) [7,8]. Data was analyzed using IBM SPSS Statistics 25. Correlations between continuous variables were studied by Pearson and Spearman correlation coefficients. The latter was added as it is less sensitive to outliers and more suitable for non-linear correlations, and there was no way to be sure in advance that a correlation, if present, would be linear. To correct for interfering variables, partial correlations were added. Means between groups were compared by T test. For correlations, a cut-off value of $p = 0.01$ was used for statistical significance as we assessed 5 main correlations and Bonferroni correction dictates to divide the standard cut-off by the number of correlations assessed. For T-test, means, p-value and 95% confidence intervals (95% CI) are reported.

Results

160 patients met the inclusion criteria described above. Among them were 97 adults (age 18 or higher), 43 adolescents (13–17 years) and 20 children (6–12 years). Baseline characteristics are shown in Table 1.

No correlation was found between age and baseline sweat chloride concentration. Moreover, no correlation was found in this group between response in sweat chloride and change in $ppFEV_1$ (Fig. 1), and between sweat chloride response and BMI change (Fig. 2, Table 2a).

Table 1. Baseline data before start of lumacaftor/ivacaftor, by sex

	Male *N* = 87	**Female *N* = 73**	**Total**
	Median (range)	**Median (range)**	**Median (range)**
Age	22 (6–43)	19 (7–41)	20 (6–43)
	Mean (SD)	**Mean (SD)**	**Mean (SD)**
Sweat chloride (mmol/l)	92.0 (9.8)	93.9 (11.1)	92.9 (10.5)
ppFEV$_1$ (%)	69.3 (24.6)	72.0 (19.8)	70.5 (22.5)
BMI (kg/m2)	20.4 (3.02)	19.2 (2.4)	19.8 (2.9)
Weight (kg)	62.1 (15.6)	50.8 (12.1)	56.9 (15.1)

Figure 1 and figure 2. scatterplots of response in sweat chloride concentration at 6 months of treatment versus response in ppFEV$_1$ and response in BMI

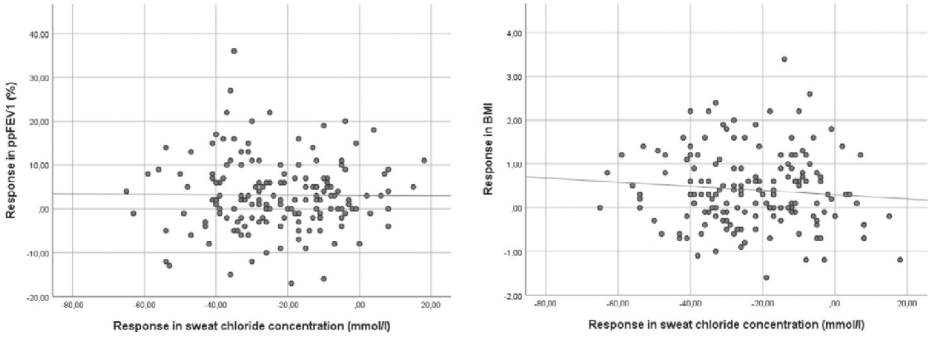


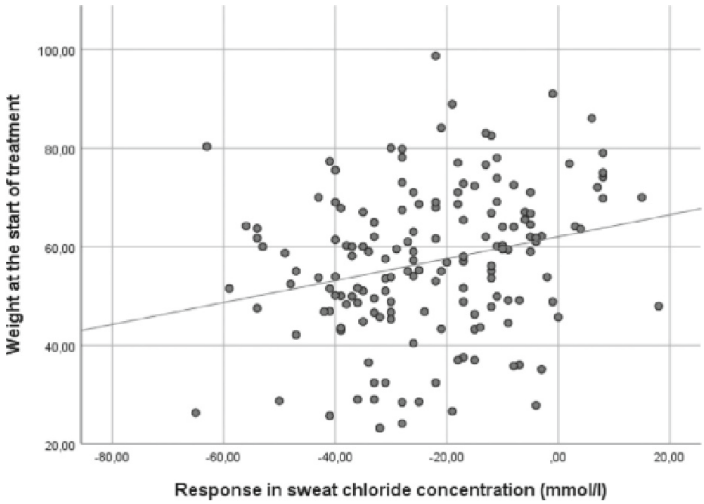
Table 2a. Correlation coefficients for continuous variables

		Pearson correlation coefficient		**Spearman correlation coefficient**	
Assessed variables		**R**	**p-value**	**R**	**p-value**
Age	Sweat chloride change	0.139	0.040	0.142	0.036
Weight	Sweat chloride change	0.244	0.001	0.252	0.002
ppFEV$_1$ response	Sweat chloride change	−0.008	0.460	−0.006	0.471
BMI response	Sweat chloride change	−0.092	0.123	−0.079	0.160
Age	ppFEV$_1$ at baseline	−0.535	<0.001	−0.550	<0.001
ppFEV$_1$ at baseline	ppFEV$_1$ response	−0.306	<0.001	−0.272	<0.001

Table 2b. Values and responses after 6 months of treatment compared between men and women

	Men		Women		Mean response difference (95% CI)	p-value
	Mean value (SD)	Mean change	Mean value (SD)	Mean change		
Sweat chloride (mmol/l)	74.0 (16.2)	−18.0	65.3 (15.0)	−28.6	10.6 (5.7–15.4)	<0.001
BMI (kg/m^2)	20.6 (3.0)	+0.28	19.8 (2.6)	+0.55	0.27 (0.01–0.54)	0.046
ppFEV$_1$ (%)	72.1 (24.0)	+2.79	75.6 (17.9)	+3.64	0.85 (−3.49–1.79)	0.511

Figure 3. Scatterplot of response in sweat chloride concentration at 6 months of treatment versus baseline weight



A negative correlation was seen between age and ppFEV$_1$ at baseline. No correlation was found between age and ppFEV$_1$ response to treatment. ppFEV$_1$ at baseline negatively correlated with ppFEV$_1$ response to treatment (Table 2a). A weak but statistically significant correlation was found between patient weight and sweat chloride change, Spearman R = 0.252, p = 0.002 (Table 2a). There was a significant difference in mean sweat chloride response to treatment between male and female patients: −28.6 mmol/l in females versus −18.0 mmol/l in males (Table 2b). Change in BMI also differed with

sex; +0.55 kg/m^2 versus +0.28 kg/m^2, this difference was also statistically significant. Difference in ppFEV$_1$ response between females and males was not statistically significant (Table 2b).

Weight did not correlate with baseline sweat chloride concentration, and no significant correlation was found between BMI and sweat chloride response or between BMI and baseline sweat chloride concentration (Table 2a). No significant correlation remains between weight and sweat chloride response with correction for age and sex: R = 0.102, p = 0.201. Similarly, the correlation between age and sweat chloride response is lost upon correction for weight and sex: R = 0.006, p = 0.937.

After correction for age and weight, the correlation between sex and sweat chloride response remains however: R = 0.253, p = 0.001. Moreover, if we assess correlation between weight and sweat chloride change within the group of males and within the group of females, we find no significant correlations.

Discussion

This study provides new information about the factors influencing sweat chloride response to lumacaftor/ivacaftor, and about the correlation of this outcome measure to other clinical parameters.

The most important findings were that sweat chloride response to treatment tends to be higher with lower weight, younger age and female sex. Partial correlations point toward sex as the key influencing factor, as the correlation between sex and sweat chloride change still stands after correction for weight and age. It also explains the correlation of sweat chloride change with weight and with age, as the mean of those two factors differed between males and females. Another relevant finding, although not the main focus of this study, is that the magnitude of the response in sweat test does not reflect the magnitude of effects with direct relevance to the patient, such as ppFEV$_1$ and BMI. This suggests that it is not only important to take into account influencing factors for sweat chloride response when interpreting these values for treatment response in practice, but should also lead to a revaluation of the sweat test as a follow up parameter in patients starting on a new CFTR modulating drug.

A key strength of this study is that the follow up data was acquired in real life practice, and recorded in a very structured way. This explains why missing data on ppFEV$_1$ and BMI did not occur in this group and missing sweat tests was sporadic; sweat test, weight and height and spirometry were all standard part of the follow up visit after 6 months of treatment. Group size is sufficient to allow reliable conclusions.

An important limitation of the study is that it will not be able to explain why the lack of correlation between sweat test and lung function response occurs. It is likely that this mainly depends on the variable extent of structural damage to the airways and lung tissue, however verification of this supposition is beyond the scope of this study.

Furthermore, we had not anticipated on the therapeutic differences between male and female patients on lumacaftor/ivacaftor. The study was not powered for studying these differences in more detail.

There have been earlier observations of a lack of correlation between sweat chloride response and FEV_1 change in the setting of ivacaftor. Durmowitz et al. studied CF patients with G551D starting treatment with ivacaftor. In this study a Pearson correlation of R=−0.003 was found in a group of 69 adolescents and adults, and R = 0.192 in a group of 22 children. Neither of the correlations were statistically significant [2]. Another study by Barry et al. led to the same conclusion [9]. In contrast, Fidler et al. did find a weak but statistically significant correlation between sweat chloride response and FEV_1 change in patients who started ivacaftor when they pooled the data form several original studies, creating a total group of 338 patients [10]. None of these studies assessed the effect of sex on sweat chloride response.

Only a few studies have explored factors influencing sweat chloride concentrations. A study by Kirk et al. involving 61 patients between 6 weeks and 37 years old, found no significant correlation between age and sweat chloride in general [11]. Cirilli et al. describe that sweat chloride concentration is not influenced by menstrual cycle, puberty state or sex [12]. Comorbidities such as adrenal insufficiency, hypothyroidism and severe malnutrition will have an effect on sweat chloride concentration and may cause falsely high results, however these conditions are quite rare in this population [13]. A correlation, or lack thereof, between weight and sweat chloride response to CFTR modulators was not reported in literature. In the phase II and phase III trials for lumacaftor/ivacaftor, pharmacokinetics were tested, but data on plasma concentrations in relation to patient weight were not reported in de article or supplements [14,15]. Data about this was also not available from the SmPC about this drug [16].

Sex influences many aspects of disease in CF, among which prognosis, with a shorter life expectancy in women, although many mechanisms behind this remain unclear. This is referred to as the ‘gender gap’ [17]. Influence of sex on the virulence of Pseudomonas aeruginosa has also been described, the female sex hormone estrogen is proposed in literature as a main factor in this by influencing the ability of Pseudomonas to produce biofilm, but also alters immune response and epithelial repair [18,19].

The differences in clinical response to lumacaftor/ivacaftor between male and female patients have not been described before in literature, except for a higher variation

between sweat chloride measurements that was described for male patients, and higher odds for female patients on lumacaftor/ivacaftor to discontinue treatment in the first year. Irregular and heavier menses have also been reported as side effects of the drug, and the use of any type of hormonal contraceptives is advised against with the use of lumacaftor/ivacaftor as these can be ineffective, suggesting an interaction of the drug with sex hormones [20,21]. A recent publication by Secunda et al. describes sex differences in the response to ivacaftor, and reports significant differences in sweat chloride change and BMI change between male and female patients, with larger responses in females. They did not find a significant difference in FEV_1 response, all of which is similar to the findings in our study for lumacaftor/ivacaftor [22].

The finding that patient sex and sweat chloride response is correlated warrants further studies into the basis of this difference, such as variation in plasma concentrations of the drug, to see if a difference in pharmacokinetics is the explanation for this finding. Further studies might be able to identify if there is a role of other mechanisms as well, such as differences in adherence to therapy or even influence of the drug on sex hormone levels. If this also is related to differences in clinical response, this could have implications for dosing regimens, possibly even affecting dosage of the newly emerging triple therapy.

Conclusion

In F508del homozygous CF patients starting lumacaftor/ ivacaftor therapy, sweat chloride response is larger in females compared to males, which also explains the negative correlation of weight with the response in sweat chloride concentration after start of lumacaftor/ivacaftor. Magnitude of the response in sweat test does not correlate to other clinical responses. This information can help the interpretation of sweat test results acquired for the follow up and evaluation of CFTR modulating treatments, and may lead to a critical revaluation of the sweat test in this setting. Moreover, the difference in response between males and females to lumacaftor/ivacaftor warrants further exploration of the underlying mechanisms of sex differences in response to CFTR modulators.

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CHAPTER 7

Correlation between chest CT findings and change in lung function on ivacaftor in CF patients

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ABSTRACT

Objective

This study aims to verify if the extent of structural lung damage visible on chest CT is correlated with change in $ppFEV_1$ after start of CFTR modulating therapy in CF patients.

Methods

In our retrospective observational study, we included patients with F508del/S1251N genotype starting ivacaftor treatment. All were in routine follow up. FEV_1 and BMI were recorded every 3 months. Patients all underwent chest CT before start of treatment. These CT scans were reviewed to determine Brody score. $ppFEV_1$ data was retrieved. To assess correlations, Spearman R and Pearson R tests were applied.

Results

Seven patients were included, with Brody scores between 0.41-27.26 (median: 12.41, normalized score out of 100) and $ppFEV_1$ before treatment of 63-113 (median: 77). Change in $ppFEV_1$ after 6 months of treatment ranged from -1 to +34 (median: +7). There was a significant correlation between Brody score and $ppFEV_1$ change, with a Spearman R=0.679 (p=0.029).

Conclusion

The extent of structural damage to the lungs of CF patients is positively correlated with the response in $ppFEV_1$ to ivacaftor.

Introduction

Despite the fact that Cystic Fibrosis (CF) affects various epithelia throughout the body, its effects on the airway epithelium are usually the most evident, resulting in structural damage to the airways and lung parenchyma that can be clearly visualized on computed tomography (CT) scan of the chest, which is widely used as an additional follow up tool in CF care [1].

F508del is the most common CF causing mutation, present on 66.7% of alleles in CF patients in Europe, and is even more frequent in the Netherlands[2]. It affects folding, trafficking, and function of the CFTR protein usually leading to a severe CF phenotype[3].

In patients who are compound heterozygous for F508del combined with S1251N, a gating mutation, a somewhat milder CF phenotype develops with a slower average decline in lung function compared to F508del homozygous patients. Targeted therapy for this mutated S1251N protein consists of a CFTR potentiator, increasing the open probability of the chloride channel[4]. The first available potentiator therapy is ivacaftor. The effect of ivacaftor for patients with gating mutations such as G551D and S1251N is overall better in comparison with lumacaftor/ivacaftor for F508del homozygous patients, especially on sweat chloride concentrations and lung function, but these effects vary greatly between individuals[5,6].

As the structural damage in CF lung disease involves longstanding remodeling of airways and lung parenchymal fibrosis, we do not expect this structural damage, nor its effect on FEV_1, to change after the initiation of therapy. Therefore, the aim of this study was to evaluate if the extent of structural damage to the lungs visible on chest scans is correlated with the response in $ppFEV_1$ after initiation of CFTR modulator therapy in CF patients.

Methods

We included CF patients with F508del/S1251N genotype from UMC Utrecht who had been using ivacaftor for at least 6 months. All patients had been in standardized follow up since the moment before start of the CFTR modulator: just before start, patients completed a CFQ-R, underwent pilocarpine iontophoresis sweat chloride testing and pulmonary function testing and measurement of weight and height. All of these measurements were repeated after 6 months of treatment.

A low dose high resolution (HRCT) scan of the chest, including expiration slides, was performed before the start of treatment in all patients. For this study, assessment of Brody score was performed by a board-certified chest radiologist. Brody score was

recorded as a total score, and separate scores on all subdomains[7]. Total scores and subdomain scores were normalized to a 0-100 scale.

Spirometry was carried out according to the current European Respiratory Society (ERS) guidelines using reference data from the Global Lung Function Initiative (GLI) to calculate $ppFEV_1$[8,9].

For analysis of the data, IBM SPSS 25 analysis software was used. To assess correlations, Spearman R was calculated. In addition the commonly used Pearson R was calculated. We consider the first the best applicable method for our purpose, as this method is less sensitive to outliers and suitable for non-linear correlation. To determine statistical significance, the cut-off for p-value was set at 0.05.

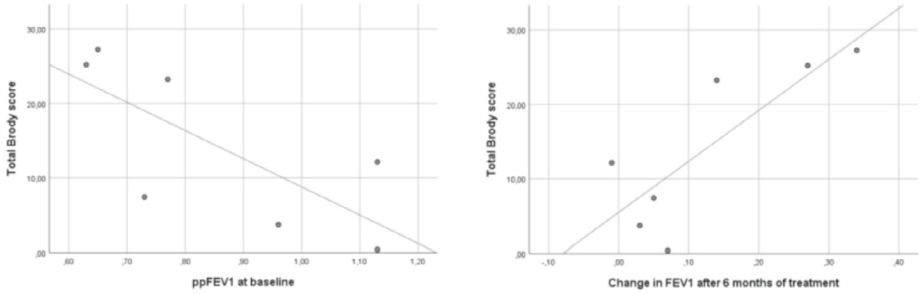
Results

Eight patients met the inclusion criteria. One patient in our center did start ivacaftor but did not complete 6 months of treatment (due to a non CF-related death) and was thus excluded. Baseline characteristics for this group are presented in table 1. Change in $ppFEV_1$ after 6 months of treatment ranged from -1 to +34 (median: +7). Brody score from baseline CT scan correlated significantly with $ppFEV_1$ at baseline, Spearman R=-0.739 (p=0.029), Pearson R=-0.734 (p=0.028). Moreover, there was a significant positive correlation between Brody score from baseline CT scan and $ppFEV_1$ change after 6 months, with a Spearman R=0.679 (p=0.029) Pearson R = 0.822 (p=0.012). Scatterplots for these correlations are shown in figure 1.

Table 1. median baseline values and range (N=7)

Age (range)	14 (9-18)
Sex (male/female)	3 male, 4 female
FEV_1 percentage predicted (range)	77 (63-113)
BMI (range)	19.0 (15.1-24.3)
Exacerbations in the year before start (range)	0 (0-4)
Brody score, total; score out of 100 (range)	12.41 (0.41-27.26)
Brody subscore: bronchiectasis; score out of 100 (range)	17.36 (0.00-34.38)
Brody subscore: mucus plugging; score out of 100 (range)	8.33 (0.00-33.33)
Brody subscore: peribronchial thickening; score out of 100 (range)	11.11 (1.85-30.09)
Brody subscore: parenchyma; score out of 100 (range)	1.85 (0.00-9.25)
Brody subscore: air trapping; score out of 100 (range)	29.63 (0.00-61.11)

Figure 1. scatterplots for Brody score versus FEV_1 at baseline and change in FEV_1 after 6 months



As Brody score in this group is correlated with both $ppFEV_1$ at baseline and $ppFEV_1$ change at 6 months, $ppFEV_1$ at baseline and after 6 months are suspected to be interconnected. Therefore we performed a post-hoc analysis calculating the correlation coefficient between $ppFEV_1$ change and Brody score, corrected for $ppFEV_1$ at baseline. The corrected correlation coefficient of R=0.576 still suggests an association, however this result is not statistically significant (p=0.231).

Discussion

This study provides a first insight into the association between extent of structural damage in the lungs and airways in people with CF and the FEV_1 response to treatment with CFTR modulators during real life follow up. There is no previous literature about this association. This study shows a positive correlation between Brody score and lung function change upon treatment with ivacaftor.

Notably, the studied group consisted of patients with a relatively well-preserved lung function and low Brody scores, reflecting only minimal structural changes in the lungs. This may explain why a positive correlation between Brody scores and lung function change on treatment was found; in patients whose lungs are not severely affected by the disease, an increase in lung function cannot be expected after the start of treatment; the goal of treatment would be to preserve lung function and to prevent lung damage rather than to reverse it. In a group of patients with more severely affected lungs, it is possible that the correlation would be absent or negative, if extensive structural damage proves to be irreversible by CFTR modulating therapy.

Our data further confirm the correlation between baseline $ppFEV_1$ and Brody score derived from chest CT, as has been established before by Helbich et al. for an earlier CT score in adult and pediatric CF patients, and by Brody et al. for Brody score in children with CF. [10,11]

The most important limitation to this study is the small group size. This makes the data more prone to be influenced by outliers, and makes it impossible to tell if the correlation found is in fact linear, or curved. Furthermore, it troubles the calculation of partial correlations, for which the study was not powered. An advantage was that in this group CT scans were timed optimally and no missing data occurred.

Verification and closer analysis of this correlation in a larger patient cohort would be favorable. Another additional approach could be to evaluate if Brody scores from follow up CT scans improve in patients while they are using CFTR modulators, and whether or not this improvement is in line with the change in $ppFEV_1$.

Conclusion

The extent of structural damage to the lungs of CF patients is correlated with the response in $ppFEV_1$ on ivacaftor. It would take a larger sample size to see if this correlation also stands after correction for baseline $ppFEV_1$. Brody score derived from chest CT in CF patients correlates with $ppFEV_1$ before the start of CFTR modulating treatment.

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CHAPTER 8

Radiological and long term clinical response to elexacaftor/tezacaftor/ivacaftor in people with cystic fibrosis with advanced lung disease

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ABSTRACT

Introduction

A triple combination of CFTR modulators ELE/TEZ/IVA (elexacaftor/tezacaftor/ivacaftor, Trikafta™) has been evaluated in clinical trials for people with cystic fibrosis (pwCF) and was approved to the European and US market. During the time between registration and settling reimbursement in Europe, it could be requested on a compassionate use basis, for patients with advanced lung disease ($ppFEV_1$<40).

Aim

The aim of this study is to evaluate two years of experience with the clinical and radiological response of ELE/TEZ/IVA in pwCF in a compassionate use setting.

Methods

pwCF who started ELE/TEZ/IVA in a compassionate use setting were prospectively followed with assessment of spirometry, BMI, chest CT, CFQ-R and sweat chloride concentration (SCC) before start and after 3 months. Furthermore, spirometry, sputum cultures and BMI were repeated after 1, 6, 12, 18 and 24 months.

Results

18 patients were eligible for this evaluation, 9 with F508del/F508del genotype (eight of whom were using dual CFTR modulators) and 9 with F508del/minimal function mutation. After 3 months, mean change in SCC was -44.9 (p=<0.001), together with significant improvement in CT (change in Brody score: -28.27 p=<0.001) and CFQ-R results (change in respiratory domain: +18.8, p=0.002). After 24 months $ppFEV_1$ change was +8.89 (p=0.002), BMI had improved by +1.53kg/m^2 (p=<0.001) and exacerbation rate declined from 5.94 in 24 months before start to 1.17 (p=<0.001) in the 24 months after.

Conclusion

pwCF with advanced lung disease experience relevant clinical benefit after 2 years of treatment with ELE/TEZ/IVA in a compassionate use setting. Structural lung damage, quality of life, exacerbation rate and BMI improved significantly with treatment. Gain in $ppFEV_1$ is lower compared to the phase III trials that included younger patients with moderately affected lung function.

Background

The discovery of CFTR (Cystic Fibrosis Transmembrane conductance Regulator) modulating molecules has heralded a new era in the treatment of cystic fibrosis (CF), as it is the first therapeutic option that targets the defective CFTR protein, instead of management of complications due to the lack of CFTR function. After ivacaftor (IVA), ivacaftor/lumacaftor (IVA/LUM) and ivacaftor/tezacaftor (IVA/TEZ), the triple combination drug elexacaftor/tezacaftor/ivacaftor (ELE/TEZ/IVA) is a new step in providing effective treatment on the protein level for an increasing number of CF patients. In the phase III trials for this drug, it proved to be highly effective in improving lung function, reducing sweat chloride concentration, and improving quality of life (respiratory domain). In these trials, patients with ppFEV$_1$ between 40 and 70 without recent exacerbations were included. Outcomes for F508del homozygous pwCF and for pwCF with F508del in combination with another minimal function CFTR mutation were comparable. [1,2,3]

During the time between registration and settling reimbursement, which applies to the situation in Europe but not in the US, it could only be prescribed after an application for a compassionate use program, via the manufacturer of the drug, Vertex Pharmaceuticals. For this program, one of the main criteria was that ppFEV$_1$ must be below 40. Therefore, patients treated in this manner were part of a group with advanced lung disease, for whom the effectivity of this treatment was not established thus far.

The aim of this study is to evaluate the clinical response and adverse effects of 2 years ELE/TEZ/IVA therapy in CF patients with severe lung damage, and to explore if highly effective CFTR modulating treatment can reverse structural changes as seen on chest CT.

Methods

pwCF treated in the University Medical Center in Utrecht, who started ELE/TEZ/IVA in a compassionate use setting between September 2019 and September 2020 were included for this observational cohort study. Persons were excluded from this evaluation if they chose not to provide informed consent for the use of their clinical data for research, if they were not able to adhere to the therapy or follow up, if they had comorbidity (e.g. end stage renal of liver failure) that had a bigger impact on their prognosis than their CF, or if they had used the drug for shorter than three months. The study was approved by the medical ethical committee in Utrecht.

All included patients were prospectively followed with assessment of spirometry, weight and height, chest CT, CFQ-R and sweat chloride concentration (SCC) before start and

after 3 months. Furthermore, spirometry and measurement of weight and height were repeated after 1, 6, 12 18 and 24 months. Sputum cultures were repeated every 6 months provided that there was a productive cough. Sputum induction of pharyngeal swabs were not performed.

Spirometry was carried out according to the current European Respiratory Society (ERS) guidelines using reference data from the Global Lung Function Initiative (GLI) to calculate the $ppFEV_1$. [4, 5] Sweat chloride concentration was measured using argentometry, after induced sweat collection using pilocarpine gel disks and Macroduct (EliTech group). From weight and height measurements, BMI was calculated for each visit as a measure for nutritional status. Chest CT was a low dose CT of the thorax without iodine contrast, and to reduce radiation as much as possible, it was performed without expiration slides. Brody score was reported by a board certified radiologist with multiple years of experience in a CF center.

The data was analyzed using IBM SPSS Statistics 23 analysis software. Mean value and standard deviations (SD) were calculated for all baseline values. Paired T-test was used to compare the measured data at baseline to those at the subsequent follow up visits. For exacerbation and hospitalization rates, the follow up duration was compared to the same length period before start. To determine statistical significance, the cut-off for p-value was set at < 0.05.

Results

Between September 2019 and March 2020, 21 patients started treatment on a compassionate use basis. Eighteen of these patients were included in our analysis. Three patients met the exclusion criteria, one due to receiving a lung transplant within the first month of treatment and discontinuing the treatment as a consequence, one was excluded in advance due to comorbidity (end stage renal failure) that was more limiting to the quality and length of life than CF itself, one suffered from pre-existent psychosocial problems that proved to make sweat test, chest CT and frequent hospital visits with spirometry unfeasible.

Data was normally distributed. Baseline values are shown in table 1. Of the nine F508del/F508del patients, 6 were previously on LUM/IVA treatment, two were on TEZ/IVA and one was not on CFTR modulating treatment because of earlier side effects of LUM/IVA. Of the nine F508del/MF patients, one was on LUM/IVA treatment on a compassionate use basis, the others were not on CFTR modulating drugs.

Treatment was well tolerated, no adverse events were seen that could be attributed to the treatment and none of the patients stopped treatment due to possible side effects.

Clinical effects of treatment and effects on Brody scores derived from chest CT scans are shown in table 2 and 3.

Three patients were previously on the waiting list for lung transplantation. All three of them were placed 'on hold' during treatment expecting later removal from the waiting list, due to improvement in their clinical status after the start of effective CFTR modulators. Two other patients had an indication for lung transplant, but were not on the waiting list. One had been rejected due to a very poor nutritional status, the other had declined lung transplant on principle. Both patients did not have an actual indication for lung transplant after 18 months of treatment.

In the 24 months before start of therapy, sputum cultures showed colonization with P. aeruginosa in 15 patients, with S. Aureus in 9 patients, M. abscessus in 1 patient, and gram negative rods (e.g. P. mirabilis, K. pneumoniae) in 11. In the first 24 months of treatment, no changes in colonization occurred in 6 patients, including the patient with M. abscessus colonization. P. aeruginosa disappeared in 1 patient, S aureus in 3 patients and gram negatives rods in 4 patients. Productive cough stopped entirely in 5 patients, so that no sputum for culture could be obtained after a year.

Due to the outbreak of COVID-19, for some patients the visit at 3 or 6 months was replaced by teleconsulting, and the chest CT and sweat test were postponed if necessary (with a maximum delay of 3 months). For some patients, spirometry after 3 or 6 months is missing because of this, and in other patients, spirometry after 12 months is missing due to COVID restrictions. In that case, BMI was calculated from home-measured weight.

Table 1. Baseline values mean and (SD), full group and divided by genotype

	F508del/F508del N=9	**F508del/MF N=9**	**Total group N=18**
Sex	8 M, 1F	3 M, 6 F	11 M, 7 F
Age	33.22 (6.70)	34.89 (14.91)	34.06 (11.25)
ppFEV$_1$	28.67 (2.50)	32.44 (6.06)	30.56 (4.90)
BMI (kg/m^2)	21.21 (3.24)	21.38 (3.97)	21.29 (3.52)
Sweat chloride (mmol/l)	67.22 (23.78)*	95.00 (12.66)**	81.11 (23.36)
Brody Score:			
Bronchiectasis	38.50 (13.89)	48.31 (17.75)	43.40 (16.21)
Mucus plugging	40.62 (19.07)	42.71 (13.60)	41.67 (16.04)
Peribronchial thickening	32.41 (10.39)	40.92 (11.78)	36.66 (11.59)
Parenchyma	5.09 (4.51)	8.33 (6.34)	6.71 (5.57)

Table 1. *(Continued)*

	F508del/F508del N=9	F508del/MF N=9	Total group N=18
Total Brody score (without air trapping score)	116.62 (37.00)	140.26 (38.32)	128.44 (38.38)
Exacerbations in 12 months before start	3.00 (2.12)	3.22 (2.54)	3.11 (2.27)
Exacerbations in 18 months before start	4.75 (3.49)	4.56 (3.75)	4.65 (3.52)
Hospitalizations in 12 months before start	2.11 (1.83)	1.33 (1.50)	1.72 (1.67)
Hospitalizations in 18 months before start	3.00 (2.73)	2.33 (2.40)	2.65 (2.50)

* Measured during treatment with lumacaftor/ivacaftor or tezacaftor/ivacaftor in 8 patients.
** Measured during treatment with lumacaftor/ivacaftor in one patient.

Table 2. Effects on ppFEV$_1$, BMI, sweat chloride (mean change, SD, and p-value)

	3 months N=10	6 months N=15	12 months N= 15	18 months N=17	24 months N=18
FEV$_1$	+5.30 (4.81) p=0.007	+8.47 (5.18) p<0.001	+7.31 (2.18) p=0.004	+9.12 (4.44) p<0.001	+8.89 (6.17) p=0.002
	3 months N=17*	**6 months N=18***	**12 months N= 15**	**18 months N=17**	**24 months N=18**
BMI	+1.13 (0.65) p<0.001	+1.34 (1.21) p<0.001	+1.83 (3.67) p=0.064	+1.60 (0.36) p<0.001	+1.53 (1.83) p<0.001
Sweat chloride (mmol/l)	-44.61 (13.50) p<0.001				
Exacerbations change		-1.72 (1.36) p<0.001	-2.50 (2.01) p<0.001	-3.77 (3.19) p<0.001	-4.77 (3.25) p<0.001
Hospitalizations change		-0.94 (0.94) p=0.001	-1.39 (1.50) p=0.001	-2.39 (2.28) p<0.001	-3.11 (2.87) P<0.001

BMI based on home measured weight in 7 patients at 3 months, and in 3 patients at 6 months.

Table 3. Effects on Brody score (structural lung damage): change in score, SD, p-value. (N=16)

Brody score domain	Change in score (0-100 scale)	p-value
Bronchiectasis	0.00 (0.51)	1.000
Mucus plugging	-13.89 (13.34)	0.001
Peribronchial thickening	-12.99 (10.10)	<0.001
Parenchyma	-1.39 (1.97)	0.013
Total Brody score (without air trapping score)	-28.27 (20.92)	<0.001

Figure 1. CT scan images of one of our patients 1 month before start (left) and 3 months after start of treatment (right), showing decline in mucusplugging and peribronchial thickening

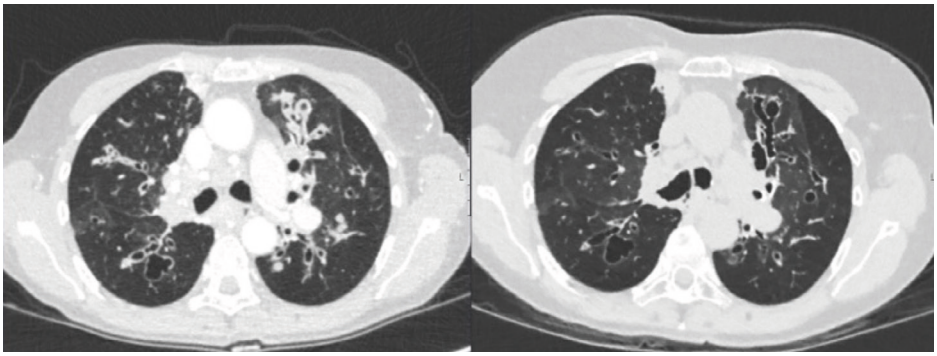

Table 4. Effects on CFQ-R (Quality of Life); changes in score, SD, P-value (N=13)

Domain	Baseline score on 0-100 scale (SD)	Change in score on 0-100 scale	p-value
Physical	50.32 (21.68)	22.12	0.001
Vitality	52.56 (23.91)	22.44	0.001
Emotion	70.77 (22.53)	7.18	0.089
Eat	88.89 (22.22)	7.69	0.108
Treat	58.12 (25.32)	9.40	0.051
Health	34.19 (28.13)	17.95	0.019
Social	66.24 (14.67)	8.12	0.089
Body	87.18 (21.20)	6.84	0.231
Role	68.59 (19.29)	12.82	0.013
Weight	79.49 (37.36)	7.69	0.553
Respiratory	63.67 (25.22)	18.80	0.002
Digestive	83.76 (11.67)	-3.42	0.264

Discussion

This study shows improvement in lung function, BMI, quality of life, exacerbation rate, sputum cultures and radiological features of CF lung disease in pwCF with advanced lung disease after initiation of ELE/TEZ/IVA on a compassionate use basis, in a real life setting. It reports clinical effects with a two year follow up duration.

It shows that mucus plugging and peribronchial thickening as seen on CT chest are largely reversible on treatment in this patient group. It further confirms that these patients have relevant benefits from the treatment, although effects on lung function are smaller compared to the patients with moderate lung disease, who were enrolled in the phase III trials. This is likely due to the larger extent and longer duration of their structural lung damage. We expect that the effects seen in our study group will be generalizable to other patients starting ELE/TEZ/IVA with comparable disease characteristics, such as poor FEV_1 and high exacerbation rate.

Newly provided information through this study, is that some of the changes in the lungs seen on CT thorax are reversible upon treatment, even after a short treatment duration. Another key finding is that improvement in the quality of life is not limited to the respiratory domain in the CFQ-R, but also the domains physical, vitality, health and role. Treatment effects on these non-respiratory domains have only once been specified before in literature, for patients with $ppFEV_1$ between 40 and 90. [6] A minimal clinically important difference (MCID) has only been reported for the respiratory domain so far. [7] It would be helpful to investigate MCID for the other CFQ-R domains in the future, as this may help in the follow up of pwCF starting new treatments. Other relevant additions to recently available studies on follow up of patients with ELE/TEZ/IVA and advanced lung damage is the two years duration of follow up and data on the change in sputum cultures.

In this two year period of follow up, we first see an evident rise in BMI, which is less pronounced on the longer term. One of the explanations for this is that patients, after experiencing their progressive weight gain in the first months, actively started to work on stabilizing their weight rather than gaining more. The majority of patients adapted their caloric intake to their new needs, and started to be more physically active around this time, while they experienced more opportunity to exercise with the gain in energy and FEV_1. This study was not set up to quantify these changes in caloric intake and exercise duration and frequency.

A few previous publications have focused on the effects of ELE/TEZ/IVA in patients with severe lung damage. O' Shea et al. report about the effects in a group of 14 patients in Ireland after 1-8 months of treatment. $ppFEV_1$ change after a month (mean 26.4 days) in this study was +9%, BMI improved by 1.4kg/m^2 after 2 months (mean

62 days). These effects seem slightly more favorable compared to those found in this study, however, comparability is limited due to differences in baseline characteristics between the studied groups, as well as the follow up duration between the studies. [8] Burgel et al. describe a larger group of patients receiving ELE/TEZ/IVA treatment on a compassionate use basis, but focus on short term outcomes (three months), so that not all outcomes in our study are comparable to those in this publication. Effects on the short term were somewhat better with respect to FEV_1 change in the study by Burgel et al. while the change in weight was more pronounced in our study. [9] Bermingam et al. describe a group of 50 patients also focusing on short term outcomes (mean follow up 39 days), finding improvement in $ppFEV_1$ of 8% and a decline in the number of patients who were referred of evaluated for lung transplantation. [10] A study by Carnovale et al. with 12 months follow up focuses mainly on nutritional outcomes in pwCF starting ELE/TEZ/IVA, but they also report FEV_1 change (mean change +10.9%, after 3 months, and +14.3 after 12 months). The publication leaves unclear if FEV_1 is reported as percentage predicted, making comparison to the effects found in our study troublesome. For BMI, a mean change of 2.0 points (3 months) and 3.1 point (12 months) was reported, which is more than in our study group. The lower BMI at baseline might be a factor in this. [11] Martin et al. studied effects of ELE/TEZ/IVA in lung transplant candidates and included 65 pwCF with 12 months follow up. Baseline $ppFEV_1$ (mean 25.0) was lower compared to the group we studied, and the reported change in $ppFEV_1$ was surprisingly large (13.4 after 1 month). The study reports a reduction in hospitalizations that is comparable to the change seen in our study. [12] In the phase III trial for the drug in compound heterozygous patients, a subanalysis was performed for the group whose $ppFEV_1$ fell under 40 during roll-in period (N=18 treatment group, N=16 placebo). In this group, effects on FEV_1 only were reported for the first 24 weeks of treatment, finding a difference of 15.8% between the placebo and treated group, which is similar to the effect for pwCF in this trial with $ppFEV_1$>40. [2] Comparability to the group we have studied may be limited as the group reported by Middleton et al. were younger patients, who until recently had a $ppFEV_1$ above 40, which differs from the group we have studied. One study with a longer follow up duration was published by McCoy et al., who followed eighteen patients for two years. Just as in our study, they described all patients reaching clinical stability while the drug was well tolerated. [13]

Inclusion criteria for both phase III trials for ELE/TEZ/IVA included $ppFEV_1$ above 40, so that the studied groups are not easily comparable to ours. For effects on FEV_1, both the trial including homozygous F508del pwCF and the one including F508del/MF pwCF found a larger difference after initiation of the treatment compared to the increase in our study. However, effects on sweat chloride and CFQ-R respiratory domain are similar to our findings. Reduction in exacerbation rate was even more evident in our study compared to the phase III trials, possibly due to the higher baseline exacerbation rate in the group with severe lung damage. [1,2,3]

A recent study by Bec et al. reports changes in Brody score on CT scans before start of ELE/TEZ/IVA and after one year of treatment. Twelve patients were included and decrease in the scores for mucous plugging (mean change -7) and peribronchial thickening (mean change -9) was found. No change in parenchyma score was found, whereas our study did find a small but statistically significant change, and found a larger change in mucous plugging and peribronchial thickening scores. Important differences between our study and the one by Bec et al. are group size, baseline Brody scores and treatment duration before follow up CT. [14] For IVA and LUM/IVA, two studies are available that used Brody scores. Sheikh et al. report Brody scores in pwCF with G551D mutation on IVA, comparing CT scans before start and after 12 months. They report a similar improvement in total Brody score compared to our study, however, they do find improvement in bronchiectasis score, and less improvement in mucus plugging and peribronchial thickening scores. Possibly, the lower baseline Brody score for the patients starting IVA and the longer treatment period before repeating CT scan play a role in this. [15] Arnaud et al. assessed Brody scores of CT scans in pwCF homozygous for F508del on LUM/IVA, comparing scans before start and after a minimum of 6 months. Reduction in Brody total score and subdomains is markedly smaller compared to our study, probably due to the smaller effect size of LUM/IVA as well as lower baseline Brody scores in the studied group. [16]

Quality of life was specifically addressed in a study by Martin et al., which reports the follow up of pwCF with severe lung disease starting ELE/TEZ/IVA, and used a newly developed questionnaire to assess impact of treatment on persons quality of life and health perception. Changes in respiratory symptoms, physical self-esteem, sleep quality were reported. [17] A minimal clinically important difference was not yet established for the used questionnaire, and no CFQ-R was performed so that the effects cannot be compared to those in our study. Fajac et al. did report effects of ELE/TEZ/IVA on CFQ-R with attention to other domains that the respiratory domain only. They found slightly more positive effects on digestive symptoms and weight compared to our study, but far less pronounced effects on physical functioning, vitality, health perception and role functioning. [6] The latter might be explained by the differences in included patients, with more severely affected patients in the group we have studied, which also reflects in lower baseline scores on most of the CFQ-R domains, leaving more room for improvement on therapy.

A limitation to this study is the relatively small group size. Although slightly larger than the previously described group using ELE/TEZ/IVA on compassionate use basis, this group size may still lead to a loss of significance in some of the outcome measures, as well as a higher vulnerability to missing data, which is an inherent challenge to this type of real-life study. Although missing data further affects group size, it is not expected to introduce bias, mainly because of the use of paired T-tests; data of a certain time point was only compared to baseline data of those same patients, rather than the whole

group at baseline. A strength of the study is he use of real-life data concerning a patient group which was not included in the extensive phase III trials for this drug.

Conclusion

pwCF with advanced lung disease experience relevant clinical benefit to treatment with ELE/TEZ/IVA in a compassionate use setting. Gain in ppFEV$_1$ is lower compared to the phase III trials that included patients with moderately affected lung function, and similar to most published studies in pwCF with severely affected lungs. Benefits on other outcome parameters including quality of life scores are similar to those in the phase III trials, BMI rises significantly with treatment and this study also demonstrates effects on structural lung damage, even on a relatively short term.

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CHAPTER 9

Management of individual patient expectations when starting with highly effective CFTR modulators

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ABSTRACT

Highly effective CFTR modulators such as elexacaftor/tezacaftor/ivacaftor (ELE/TEZ/IVA) will become available for an increasing number of people with cystic fibrosis (pwCF) in the near future. Before the start of this therapy, many questions may arise concerning the expected effects. We assembled the currently available data from the literature about ELE/TEZ/IVA that focused on commonly asked questions from patients. Overall, the literature so far presents a very hopeful prospect of effects, not only on lung function, but also on nutritional status, sinonasal symptoms and quality of life. The effects in patients with pwCF with severe lung damage are also favorable. Treatment is generally well tolerated. In some cases, patient-derived cell models can help in predicting the effects for individual patients.

Introduction

In cystic fibrosis (CF), a mutation in both alleles of the CFTR gene leads to the production of a CFTR protein that is insufficient in structure, length, quantity or stability, depending on the exact mutation. As the protein normally functions as a chloride and bicarbonate channel across the cell membrane on the apical side, an insufficiently functioning CFTR protein in turn leads to a lack of chloride and bicarbonate transport, causing problems of thick mucus and pH imbalance in multiple epithelial tissues, hereby affecting many organs including the lungs, intestines, pancreas and sweat glands [1].

Although CF is a genetic disease, the most impactful developments in recent years were not therapies targeting the defective gene, but rather the resulting protein [2–5]. For an increasing number of mutations, this leads to a significant improvement in protein function. The class of small molecule drugs targeting the CFTR protein in order to restore functionality is called the CFTR modulators. The first to become available was the potentiator molecule ivacaftor, which acts on the CFTR protein affected by gating mutations in which it increases open channel probability, thus restoring the protein's function [6–8]. It is used for CFTR mutations such as G551D and S1251N [9].

A second CFTR treatment option is a combination of the mentioned ivacaftor with a corrector, lumacaftor. Lumacaftor acts on protein processing in the cell leading to a larger proportion of the produced protein reaching the cell membrane instead of being degraded, where ivacaftor can then enhance the channeling function. This combination treatment is suitable for a larger number of people with CF (pwCF), as all pwCF homozygous for F508del are eligible; however, it is much less effective compared to ivacaftor for pwCF with suitable mutations [10]. A newer combination, tezacaftor/ivacaftor, was later added as a treatment option with similar efficacy to lumacaftor/ivacaftor, with slightly less side effects and less interactions with other drugs [11].

New hope arises with the emergence of triple therapy, of which the first available combination is elexacaftor/tezacaftor/ivacaftor (ELE/TEZ/IVA, Vertex therapeutics®). In phase III trials, this treatment was shown to have a drastic positive effect on clinical parameters such as lung function, pulmonary exacerbations, CFQ-R and sweat chloride concentration [12,13]. ELE/TEZ/IVA is currently accepted in the market for the treatment of patients with at least one F508del allele. In vitro studies, however, have also shown that this combination drug improved CFTR function in selected non-F508del genotypes [14–17]. However, with this new hope, many questions will arise concerning individual expectations surrounding the start of therapy. This review will not be able to address all of the related insecurities encountered in the CF clinic, but it aims to provide an overview of the current evidence concerning some frequent and relevant questions about expectations of the effects of newly started CFTR modulators. For many patients,

this new modulator will be ELE/TEZ/IVA, sometimes switching from treatment with LUM/IVA (lumacaftor/ivacaftor), TEZ/IVA (tezacaftor/ivacaftor) or IVA (ivacaftor).

Questions to Address

1. My lungs are severely damaged already. Will I still benefit from this drug?

It is expected that CFTR modulator therapy is most effective if initiated early, before structural damage to the lungs has occurred. However, in the current practice, many patients starting ELE/TEZ/IVA therapy will already have longstanding structural lung damage. Although patients with severe lung damage resulting in a predicted FEV_1 below 40% were not included in the phase III trials for the drug, there are clinical data available about the effects for this group of patients.

O'Shea et al. describe a group of 14 patients enrolled in the managed access program for ELE/TEZ/IVA, all with a $ppFEV_1$ below 40 percent. At one month follow up, the mean $ppFEV_1$ improved by 9.0%, and the mean BMI change was +1.7 kg/m^2. Both results were statistically significant despite the small group size [18]. In the phase III trial published by Middleton et al., a subgroup was identified of 18 patients whose lung function declined to values below a $ppFEV_1$ of 40% between inclusion and the start of treatment. After four weeks, the mean difference FEV_1 was 15.2% compared to the placebo [13] The effects on lung function in this group are therefore comparable to the results in the phase III trials, in which patients were required to have $ppFEV_1$ of 40–90% before the start of treatment. Both these studies were focused on short-term outcomes. A larger group of pwCF with advanced lung disease starting ELE/TEZ/IVA was evaluated by Burgel et al. The follow-up duration was 9 months, longer compared to the other studies. Gain in lung function was comparable to that in the phase III trials, and additional effects on the need for supplemental oxygen and decrease in enlistment for transplantation were reported [19].

There are no studies concerning the effects of ELE/TEZ/IVA on severely affected lungs in the longer term yet. For pwCF with moderately affected lungs ($ppFEV_1$ 40–90%), effects on lung function have been reported for the first 24 weeks of treatment, showing a mean increase in $ppFEV_1$ of 11.9% for pwCF with the F508del/F508del genotype who received TEZ/IVA earlier, to 14.9% for pwCF with the F508del genotype and a minimal function mutation who received no prior CFTR-modulating treatment [20]. To get a glance at the longer-term outcomes in pwCF with advanced lung disease, it may be useful to look at the long-term effects of ivacaftor for patients with a gating mutation and severely affected lungs, as both ivacaftor in patients with a gating mutation and ELE/TEZ/IVA for patients with at least one F508del allele have a nearly similar effect in restoring CFTR function. Taylor-Cousar et al. describe a group of 44 patients with the F508del/G551D genotype and $ppFEV_1 < 40\%$ or listed for lung transplantation. After 24 weeks of treatment, an increase in $ppFEV_1$ of 5.5% was seen and an increase in weight

of 3.3 kg [21]. Barry et al. describe 21 patients with the F508del/G551D genotype with a mean follow up of 237 days and ppFEV$_1$ < 40 or listed for transplantation, and found a FEV$_1$ change of 4.2% along with a marked reduction in exacerbations [22]. A smaller group of 14 patients with a gating mutation and ppFEV$_1$ < 40 was studied by Hebestreit et al. In this group, an improvement of ppFEV$_1$ of 5.2% was seen after a mean follow up of 34 weeks [23].

In conclusion, there is a mean improvement in FEV$_1$ after the start of highly effective CFTR modulation, even in pwCF with severely affected lungs. However, this change will be smaller compared to patients with mild or moderate lung damage. On other parameters such as BMI, the effects are similar even with advanced disease.

2. Is there any way to know in advance if my response will be better or less than average?

To get an estimation of medication effects before starting the drug in real life, the in vitro measurement of CFTR function by the use of organoids can be useful. Berkers et al. describe the in vitro effect of ivacaftor and LUM/IVA on patient-derived rectal organoids that express CFTR abundantly, which correlates with the clinical effects of the tested CFTR modulators. In specific cases, such as patients with a rare genotype, this organoid technique might help to assess if a CFTR modulator will be effective or not [24]. A recent addition to CFTR testing in patient-derived cells is the assay on cultured nasal epithelial cells. Using this method, it would be harder to test cells of many different patients at the same time, but on an individual level, it is an accurate and feasible way to assess the effect of CFTR modulators in patients cells. The method was also validated for the correlation between the in vitro response and clinical effects of CFTR modulators [3].

3. Will I gain weight?

The effects of CFTR modulators on nutritional status have been reported in a review by Bailey et al. From available studies on the respective drugs, they found a relevant BMI increase with ivacaftor for pwCF with G551D, but not with R117H. The BMI increase with TEZ/IVA was not found to be significant. For the effect of ELE/TEZ/IVA on BMI, the review based itself of the phase III trials, finding a BMI change of +1.04 kg/m^2 in pwCF heterozygous for F508del after 24 weeks of treatment, and +0.6 kg/m^2 in pwCF homozygous for F508del after 4 weeks of treatment [25].

Since then, Griese et al. have added data for the longer term and found an increase in BMI of 1.30 kg/m^2 compared to baseline for pwCF homozygous for F508del after 36 weeks, and an increase of 1.28 kg/m^2 compared to baseline for pwCF heterozygous for F508del after 48 weeks of treatment [20].

4. Will there be (severe) side effects?

Based on reporting of adverse events during the phase III trials, side effects for ELE/TEZ/IVA are infrequent and usually mild. The main reported side effects directly attributed to ELE/TEZ/IVA use are rash and elevated transaminases. During the first 24 weeks of treatment, rash occurred in 9.9–10.9% of patients, leading to discontinuation in 0.2–0.5%. Elevated transaminases occurred in 4.2–5.4% of patients, leading to discontinuation in 0–0.6% [20]. Adverse events, such as hemoptysis or pulmonary exacerbations, occurred more often in the placebo group compared to the treatment group. Cough, however, was more common in the treatment group, as well as upper respiratory tract infections [12,13].

More severe adverse events have been reported to occur incidentally, mainly through case reports, with serum sickness-like reaction, biliary disease and testicular pain as examples [26–28]. No data are available so far about the use of ELE/TEZ/IVA in patients with severe liver failure due to CFLD.

5. Will this treatment save me some hospital admissions?

There are some available data on exacerbation rates and hemoptysis after the start of ELE/TEZ/IVA, so that an estimation of reduction in hospital admission should be feasible. In the phase III study for this drug in F508del homozygous patients, exacerbation counts were low due to the short follow up of 4 weeks. In the ELE/TEZ/IVA group, exacerbations were reported in 2% of patients compared to 12% in the TEZ/IVA group, and hemoptysis in 4% versus 10% [12]. The other phase III trial that included patients with F508del and a minimal function mutation had a longer follow up time of 24 weeks. During this time, pulmonary exacerbations occurred in 21.8% of the ELE/TEZ/IVA group versus 47.3% in the TEZ/IVA group. Hemoptysis also occurred less in the ELE/TEZ/IVA group versus the TEZ/IVA group: 5.4% versus 13.9%, respectively [13].

In the prolonged phase III trial, including both F508del homozygous and compound heterozygous patients, the exacerbation rate was reported at 0.30 per 48 weeks, but this study had no control group and the exacerbation rate was not compared to that before treatment [20].

As infectious pulmonary exacerbations are the main cause for hospitalization for CF patients, it is very likely, based on the current evidence, that the use of ELE/TEZ/IVA will lead to a drastic reduction in hospital admissions.

6. Will this drug improve my sinus problems?

Chronic rhinosinusitis and nasal polyps contribute to the morbidity in people with CF, with a large impact on the quality of life. Description of the effects of ELE/TEZ/IVA on these sinonasal problems has been performed in two studies so far, both evaluating symptoms using the SNOT-22 questionnaire. DiMango et al. included 43 participants, and in this group, a reduction of the SNOT-22 score of 10.5 points was seen, from a baseline score of 34.8 [29]. Douglas et al. evaluated a group of 25 patients and found very similar effects; a score reduction of 10.2 from a baseline score of 34.5 [30].

As both studies used a validated symptom scale and found a large difference in symptom score after treatment, the conclusion should be that ELE/TEZ/IVA can significantly reduce sinonasal symptoms.

7. What is known about this drug in the context of starting a family?

As it is known, CFTR is expressed in the cervical and endometrial epithelium, causing decreased fertility in many females with cystic fibrosis; thus, it is logical that the use of a CFTR modulator can result in the increased incidence of pregnancy, which has also been described for ELE/TEZ/IVA in a case series [31]. This is, however, important to discuss before the start of treatment, because in the past with ivacaftor, this has resulted in unexpected and sometimes unwanted pregnancies. If a woman with CF gets pregnant while using CFTR modulators, it is important to weigh the benefits of the treatment to the mother versus the potential harm to the child. However, very limited data are available about the unborn child's safety during the use of CFTR modulators in the mother [32].

One recent study is available on the safety of ELE/TEZ/IVA, including 45 patients who used the medication for a median duration of 3 months during pregnancy. During the study, 29 pregnancies resulted in live birth and 7 reached the second or third trimester without complications.

Four first trimester miscarriages occurred, which is in line with usual incidence of miscarriages in the US, of which one was reported as having unknown relatedness to modulator use, and the other three were deemed unrelated. Two unintentional pregnancies were electively terminated, and one first trimester pregnancy was electively terminated because of severe malformations in a woman with poorly regulated diabetes. No exacerbation deemed related to ELE/TEZ/IVA use was reported. Five premature births occurred, none of which were deemed related to the medication. In the infants, screening for cataracts was not standard. In the four infants who underwent screening, no cataracts were found. It is known that all components of ELE/TEZ/IVA, and also of LUM/IVA, are present in breast milk if the mother takes this medication. Screening of the child for cataracts is not always done, and in the two children who were screened for this indication, no cataracts were found [33]. One child who was breast fed

while the mother was on LUM/IVA therapy had elevated transaminases by day 29 after birth, which normalized after reduction of the breast-feeding fraction and recurred with full breast feeding. Although this was just one reported case, it could be advisable to monitor transaminases in breastfed infants whose mother takes CFTR modulators [34].

8. Will I be able to do sports/work again?

Although the effects of ELE/TEZ/IVA on lung function are convincing for its effectiveness, the exact effects on daily functioning will be hard to predict, mainly because mentioned work or sports activities will vary a great deal between individual, as will their baseline condition before the start of treatment.

To have some estimation of functional gain, CFQ-R scores before and after the start of therapy can be helpful. However, most studies only report the respiratory domain, although the domains 'Role', 'Social', 'Vitality' and 'Physical functioning' will better help estimate functional gain. One study reported all CFQ-R domains (scale 0–100) for a group of 43 patients before treatment and after three months. In the 'Social' domain, the mean change in scores was +6.7. Scores in 'Role', 'Vitality' and 'Physical' functioning improved by 10.0, 12.5 and 13.3 points, respectively. A minimal clinically important difference was reported in the literature for the respiratory domain only [35].

Discussion

When starting a new CFTR modulating drug, it is of great importance to discuss expectations about the new treatments in an open manner. This helps the patient to have a realistic view of the potential effects for their situation, but also knowing about possible adverse effects will improve safety of the treatment. This review could support that by providing an overview of the now available literature. However, it will not be able to answer all individual patient questions for multiple reasons.

At this point in time, data about the effects of ELE/TEZ/IVA are very limited on all aspects of treatment, although new information is published at a high speed. In the upcoming years, there will at least be a lack of long-term data about this treatment.

In some situations where the literature about ELE/TEZ/IVA effects is lacking, it can be a good option to turn to studies performed with IVA, which also has a potent CFTR-modulating effect. It is important when reviewing this evidence that these studies specifically included patients with a gating mutation, who on average will have a better condition compared to patients with class I or II mutations at the same age. Moreover, the pharmacokinetic and pharmacodynamic properties of the combination drug are different from ivacaftor alone. Therefore, extrapolation of study results about other

CFTR modulators should be done with caution only, even when it is the best information available at the time.

To be able to inform an individual patient about the expected effects, it is not only important to have recent literature available, but also to evaluate if your patient's situation is comparable to that of the study population.

Conclusions

The current literature points towards favorable effects of ELE/TEZ/IVA on lung function as well as sinus problems and quality of life. The drug is overall well tolerated and severe adverse reactions are scarce. ELE/TEZ/IVA seems to improve fertility in female pwCF, while the treatment's continuation during pregnancy is currently advised against, due to a lack of robust data about risks to the unborn child. Cell models such as organoids or cultured nasal cells can be used to estimate in advance if effectivity of the drug can be expected. An increasing amount of data are published regarding the effects of ELE/TEZ/IVA, helping to better inform patients in the discussion of their expectations when starting new CFTR modulators. However, the literature addressing differences in the response between patient groups with selected characteristics is still very limited.

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CHAPTER 10

General discussion and future perspectives

Main findings in this thesis

In this thesis we searched for improved individual indicators of treatment effects and thus evaluated various CFTR functional assays and clinical outcome parameters to determine their relevance in the follow up of pwCF starting CFTR modulating treatment. This is highly important since the widely used primary endpoint FEV_1 in clinical trials has proven to be non-predictive of individual treatment effects in the long term.

In vivo tests that inform on CFTR function, such as sweat chloride and NPD that we used in the evaluation of patients with 5T in chapter two, remain essential in diagnosing CF, but tests of CFTR function can also help to estimate or even predict the effects of CFTR modulating treatment before in vivo application. In chapter three, we developed such a complementary in vitro approach using Ussing measurements in cultured patient-derived nasal cells. In vitro CFTR function tests are an important addition to clinical parameters, as they can be tailored in vitro to specifically and precisely measure CFTR using defined in vitro conditions (e.g. by manipulation through inhibitors of culture conditions) and enable large scale testing of conditions. The Ussing chamber measurements in patient derived nasal cells can be a convenient, patient friendly addition to rectal organoids which are currently under development and standardization, and may potentially reflect airway physiology and benefit better than intestinal cells.

In addition to lung function and pulmonary exacerbation rate, BMI, CFQ, CT and sweat test are important outcome measures in the follow up of pwCF starting new CFTR modulating treatment. The sweat test was evaluated in chapter 6, finding that sex appears to influence the magnitude of sweat chloride response to treatment. Chest CT was explored as an outcome parameter in chapters 7 and 8, where it was shown how CT Brody scores change upon treatment. Thus far, we could not confirm that this parameter was correlated to other clinical outcomes, possibly due to the small group size. Combining various measures provides a more accurate view on the treatment effects compared to FEV_1 alone, particularly in patients with a well-preserved or severely affected lung function. This is illustrated in chapters 5 and 8.

Questions from a patients' perspective surrounding the start of highly effective CFTR modulators were addressed in chapter 9. The answers are based on the most actual available evidence at the time, and aimed to aid clinicians. The in vivo and in vitro measurements of treatment effects come together in cases such as the one described in chapter four, where in vitro techniques were instrumental in advocating eligibility for the drug, and the clinical outcome parameters together provide a comprehensive overview of the massive impact of the treatment to the individual patient.

Expanding clinical follow up modalities in patients starting CFTR modulators

Since the establishment of clinical studies for highly effective CFTR modulators, we have gained a deeper understanding of how various outcome parameters relate at the population level. The phase III trials for IVA/TEZ/ELE provide a comprehensive overview of commonly used follow up parameters around the start of new CFTR modulating treatment. $ppFEV_1$ has long been a central parameter, partly because it is routinely used in CF patient follow up and has demonstrated a correlation with prognosis in the past. While it remains a central follow up parameter, this thesis is focused mainly on supplementary outcomes as FEV_1 is not the best parameter for every patient group. This was illustrated in chapters 5 and 8, which describe pwCF with well-preserved lung function and pwCF with severe lung damage, respectively. In these groups, smaller treatment effect on FEV_1 can be expected but effects on other parameters were similar to those found in the phase III clinical trials. This apparent lack of relation between FEV_1 and other organ-specific outcomes indicate the need for a more detailed study of how CFTR functions within different organs, and how this leads to individual disease and modulator response.

Exacerbation rate is an important second outcome measure in studies with sufficient follow up duration, as it has a strong correlation with lung function decline over time and quality of life. It has been used in some of the large phase III trials for CFTR modulators, and in our study in chapter 8, among others, it has been a very relevant outcome as the steep reduction in exacerbations signified an important impact on quality of life. Additionally, sweat chloride concentration, BMI and CFQ-R respiratory domain are common outcome parameters. These are frequently used in follow up around the start of treatment outside of clinical trials. These were also used in the (extended) phase III trials and show clear, quantitative correlations on population level. [1, 2, 3] In this thesis, these outcome parameters have been used to reach a more complete analysis of efficacy of treatment among different patient groups. It varies per patient group which parameters are most important.

Recently, interesting findings were published by Sadras et al. about the clinical response to IVA/TEZ/ELE in patients with F508del/N1303K and N1303K/N1303K. It was an observational study of 8 patients who started off label therapy with IVA/TEZ/ELE. Sweat test response was not significant in these patients, while responses in $ppFEV_1$ (+18.4) and BMI (+0.79kg/m^2) were comparable to other F508del/MF patients. [4] These unexpected findings raise new questions about the differences in treatment response across tissues, suggesting variations in CFTR expression between different tissues in the body. Although these findings remain unexplained in this study, it shows that outcome parameter are not always in agreement, and supports the evaluation of multiple outcome parameters to evaluate response. Moreover, it leaves relevant questions concerning the reason for this apparent mutation-associated, tissue-specific response to the medication. It remains unclear whether this is attributable to variations

in tissue penetration, differences in CFTR expression throughout the tissues or other contributing factors. Further investigation into these aspects could provide important insights in the variability of CFTR modulator treatment effects.

When CFQ-R is used as a clinical outcome parameter, usually only the respiratory domain is reported. However, the other domains of the CFQ-R should also be used in the follow up of patients starting IVA/TEZ/ELE. These are a logical addition as the CFQ-R questionnaire is performed as a whole and not as separate questionnaires per domain. It comes as no surprise that effective treatment influences almost all domains of the CFQ-R instead of the respiratory domain alone. However, for the other domains a MCID (minimal clinically important difference) had not yet been established which makes interpretation of the differences complicated. [5]

An additional, less commonly used outcome parameter in the follow up of CFTR modulating therapy is cardiopulmonary exercise testing (CPET). This was not included in the studies in this thesis. It has thus far been used in small studies concerning ivacaftor and IVA/TEZ/ELE, showing increase in VO_2peak and VO_2peak/kg after start of treatment, indicating a better physical condition. [6, 7] CPET seems quite labor intensive to apply for all patients starting treatment, but could contribute to the insight in overall effects of the treatment on a group level and might be of significance in individual patients where lung function improvement is lacking and other recordable effects are warranted.

To conclude, relations between CFTR function and organ-specific function measurements in the context of long-term benefit of CFTR modulator treatment remain unclear. Current data support the further exploration of parameters beyond spirometry. This would improve our understanding of organ-specific disease and treatment outcomes, and how these reflect individual phenotypes. The studies in this thesis support a flexible approach, adjusting the follow up parameters to the specific patient characteristics and always combining multiple modalities.

Patient-specific cell models as in vitro tool for testing efficacy of CFTR modulators

Airway cells cultured from individual pwCF have been studied and reported in the literature by various research groups, each using different methods for cell culture and CFTR function measurement. Whole cell current measurement has been performed on both fresh and cultured human nasal epithelial cells (HNECs), demonstrating that culturing the cells had no effect on CFTR expression and function. Measurements correlated well with Ussing measurements of cultured cells on filter, suggesting that differentiation of the cells in ALI do not impair CFTR expression. [8, 9] Ussing measurements have been performed after culturing the cells in several ways, with differences in conditional reprogramming and expansion of the cells, yielding quite similar results in CFTR function. [10, 11] The approach to conditional reprogramming

and expansion used in this thesis is also different from those previously described in literature, yielding similar results however when CFTR function is measured.

CFTR function can also be measured in nasal organoids, where HNECs are first cultured in a similar way to the approach for Ussing measurements, but instead of leaving them on filters, the monolayer of cells is disrupted so that the cells form spheres. More recently, methods have also been published to culture airway organoids without culturing the cells in ALI first. [12] If CFTR is stimulated and chloride transport is induced, water follows into the lumen of the spheres causing swelling. The amount of forskolin-induced swelling (FIS) is then the readout for CFTR function. [13,14] This method has analogies to the longer and more widely used approach of measuring CFTR function through FIS in rectal organoids, which has reached more standardization. [15]

Compared to nasal organoids, rectal organoids provide a cleaner readout for CFTR function as forskolin-induced swelling is fully CFTR dependent. In nasal epithelial cell cultures, other ion channels are present as well and CFTR is expressed in smaller amounts, so that CFTR-independent mediated chloride transport will also impact on the assay outcome. This makes the nasal epithelium more complex to use as a readout for CFTR function, but in other ways more versatile as it may also be used to evaluate the function of other ion channels in the airway epithelium.

Future perspectives and recommendations for further research

As we move forward towards a more comprehensive use of clinical outcome measures and even in vitro models for patient-specific phenotyping and CFTR function estimation, many steps remain needed to further refine and validate the methods so they can be optimally used for clinical applications.

For in vitro methods, a new opportunity would be to validate CFTR measurement on HNECs as a diagnostic measurement. For a proportion of people for who current diagnostics are inconclusive, in vitro outcomes in nasal cells should be compared to established diagnostic criteria and other tests of CFTR function such as NPD or ICM measurements that are currently used to phenotype inconclusive diagnosis. For these CFTR functional measurements, test repeatability and reproducibility, test sensitivity and specificity for diagnosis, and patient comfort need to be compared to determine when to use which methods.

The same would apply to the use of intestinal organoids as a diagnostic tool, which are currently further in development in this direction. The most established method for this is rectal organoid morphology analysis (ROMA), an essay of roundness of the non-stimulated organoids. ROMA indices have been found to correlate with genetics, pancreatic status and sweat chloride. In an exploratory study of patients with uncertain diagnosis after genetics and sweat test, ROMA helped establish a diagnosis in 75%. [16]

Another assay in organoids that is under development for diagnostic use is steady-state lumen area (SLA), but diagnostic value for this method is not published so far. For intestinal organoids, a correlation has been identified between residual CFTR activity and prognostic factors such as annual lung function decline and development of pancreatic insufficiency, liver disease and diabetes [17]. In the future, it would be helpful to see if the same correlation applies to the nasal cells as a model.

Another step in the further applicability of the CFTR functional measurements in patient derived cells would be to establish concordance between measurements in nasal cells and in intestinal organoids: it would help the applicability of both modalities to establish that they are equally suited for use, depending on the laboratory facilities and patients' or doctors' preference.

Such in vitro models, especially when they recapitulate in vivo CF disease and therapeutic response could also play an important role to identify new treatment options for those people with CF who are not eligible for CFTR modulating treatment because of a non-responsive genotype. Especially while these patients already see peer patients benefitting from new treatment, there is an urgent need for this relatively small group not to be left behind. Depending on the genotype, various approaches are being evaluated currently, varying from gene therapy to read-through agents or medication targeting non-CFTR chloride channels on the epithelium. [14, 19, 20]

For the follow up of pwCF, we need to both broaden our observations to further study outcomes in many different organ systems that are affected by CF, and how disease progresses, halts or reverses in the long term upon initiation of novel CFTR modulating treatments. Specific attention to psychosocial and neurocognitive impact of the treatment, both positive and negative, is warranted. Tools are in development to monitor side effects of CFTR modulator treatment. Validating these will be important to be able to implement them as standardized screening tools in follow up, to prevent overlooking or underestimating adverse treatment effects. The validation of such tools has not been published yet. As indicated, it will be increasingly important to gain structured long term follow up data about the effects of highly effective CFTR modulators like IVA/TEZ/ELE. First of all it will take long term follow up data to explore the effects on life expectancy, but also the long term safety profile of the drugs will need to be established, as well as safety of use during pregnancy and lactation, which to date is not fully clear.

With life expectancy expanded on new therapy, another challenge in the future will be the problems ageing pwCF will face, e.g. complications of diabetes, cardiovascular disease and osteoporosis, and if such problems are addressed by systemically-acting CFTR modulators or new upcoming genetic treatments that restore CFTR only locally in the lungs. Ageing-related health problems in pwCF are currently prospectively studied

in pwCF in UMC Utrecht, among other centers. This might provide insights on how to best guide and treat pwCF to achieve optimal healthy ageing. Part of this will be to keep in close contact with the patients despite the possible less frequent need for clinic visits if stability of the disease is reached.

The cost-effectiveness of current highly effective CFTR modulators will also need further attention. It is very relevant to investigate how the current high treatment costs relate to economic benefits such as reduced hospital care (reduced clinic visits, admissions, lung transplants), and increased work participation. In the following years we will also have to consider if these treatments will prevent or lead to additional costs related to ageing of CF patients, and how costs of treatments will develop in time when patents expire.

With patients starting highly effective CFTR modulating therapy early in life, the focus in CF care will transfer more and more from tertiary prevention (preventing existing damage from further progression) to secondary prevention (preventing the development of any damage from the underlying disease). This requires a different approach to the evaluation of effectiveness of the drug, as lung function and nutritional status will be normal at the start of treatment. In addition to long term follow up to assess stability of these parameters, the sweat test will become increasingly important. It can serve as a readout for effectivity of the drug on CFTR function, which can be performed already in infants and might at that point be the only indicator of treatment effect. It allows assessment of treatment response on a short term as well as long term, in contrast to all other outcome measures that rely on indicating organ damage. Patient cells might provide an additional testing platform when more drugs are available so that drugs can be individually selected based on the highest increase in CFTR function.

Despite additional tools being developed for monitoring health related quality of life (HRQoL) in CF in the era of CFTR modulators, CFQ-R will remain an important tool to follow up on patients' HRQoL over time. To do so while starting new treatment, it would be an important addition to establish MCID for the other domains, as has been done for the respiratory domain. [17]

Concluding perspective

The studies in this thesis merely provide an exploration of a more comprehensive use of several outcome parameters for CF patients starting CFTR modulating treatment, it cannot provide any kind of prediction model for the long-term effects. With the current evidence, it pleads for the use of combined CFTR functional assays and clinical parameters instead of focusing on one central outcome only. The fact that CF disease has various expressions throughout the body and treatment effects are highly variable between individuals with CF, is a warning not to view treatment effects in 'black and white'.

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CHAPTER 11

English summary
Nederlandse samenvatting

English summary

This thesis shows how CFTR functional testing can help in estimating the effects of a certain CFTR mutation and the effects of CFTR modulators on an individual basis, and how additional clinical follow up parameters apart from lung function can help evaluations of treatment effects in the clinical setting. Chapter one provides an introduction into this subject, the aim of this thesis and the methods that were used.

In chapter two we focus on 5T, a mutation of varying clinical consequence. We used NPD, sweat test and clinical parameters to evaluate the severity of consequences of the mutation. We show that it is important in the evaluation of individuals with 5T to not only include TG repeats in the genetic evaluation, but also to make use of multiple clinical tools and signs in the evaluation of these patients, as expression of clinical problems varies greatly among patients with this splicing mutation.

In chapter three we explore how Ussing measurements of treatment effect in cultured nasal cells of individual patients correlates to clinical effects in these patients. As the in vitro and in vivo measurements are correlated, it could be a useful option for in vivo prediction of in vivo treatment effects in the future, as a patient-friendly and feasible addition to rectal organoids.

Chapter four provides a real life illustration of how various methods of CFTR functional measurement (FIS in rectal organoids and Ussing in nasal cells) as well as follow up modalities were applied in the case of an individual pwCF who was found eligible for compassionate use treatment with ELE/TEZ/IVA based on CFTR functional measurements and had a very favorable response.

In chapter five we studied a group of F508del homozygous patients starting LUM/IVA while their ppFEV_1 was 90 or higher. While their lung function hardly changed, we did see changes in BMI, sweat test, exacerbation rate and quality of life scores, suggesting that relevant effects also applied to this subset of CF patients and that it is important to focus on more than FEV_1 alone during follow up after start of modulating therapy, especially in patients with ppFEV_1 outside of the regularly studied 40-90.

Chapter six provides a closer look at another follow up parameter used with CFTR modulators: sweat chloride testing. We described the decrease in sweat chloride levels in F508del homozygous patients starting LUM/IVA. We found that age and weight did not have a relevant influence on the magnitude of sweat chloride response, however sex did; the response in females was larger than in males. In this study we did not find an explanation for this and we did not find a better response in females in terms of FEV_1 or BMI.

Chapter seven evaluates Brody scores calculated from CT scans of F508del/S1251N patients before start of ivacaftor treatment, and correlation of this score to the lung function response on treatment. We found that patients with a higher Brody score had more improvement in lung function on therapy, suggesting in this group with relatively well-preserved lungs, that the patients with most limited lung damage had less room for improvement of their lung function upon start of treatment.

Chapter eight describes a group of CF patients with severe lung damage who started ELE/TEZ/IVA on compassionate use basis, and the treatments effects on the short and longer term. A drastic response on CT scan was seen in these patients with reduction of mucus plugging and peribronchial thickening, as well as a moderate response in FEV_1 and good response in BMI and exacerbation rate. Overall this group of patients respond less in lung function, but on other outcomes just as well as CF patients starting treatment with moderately affected lungs.

Chapter nine provides an overview of relevant questions that people with CF may have before starting highly effective CFTR modulating therapy. These questions are addressed one by one with the available evidence at that time, to provide a tool for clinicians discussing commencement of therapy with their patient.

In the general discussion we compare the findings in this thesis to those in other literature, and to conclude, various evidence gaps and future research opportunities were discussed.

Nederlandse samenvatting

Dit proefschrift laat zien hoe CFTR functietesten kunnen helpen bij het inschatten van de effecten van behandeling met een CFTR modulator bij individuele patiënten, en hoe aanvullende klinische follow up parameters naast de longfunctie kunnen ondersteunen in de evaluatie van behandeleffecten. Hoofdstuk een geeft een inleiding op dit onderwerp, het onderzoeksdoel van dit proefschrift en de gebruikte methoden.

In hoofdstuk twee richten we ons op 5T, een mutatie met uiteenlopende klinische consequenties. We gebruikten NPD, zweettest en klinische NPD parameters om de ernst van de consequenties in kaart te brengen. We tonen aan dat het bij de evaluatie van personen met 5T van belang is om niet alleen TG repeats te meten, maar ook een combinatie van klinische parameters te gebruiken, aangezien de uitingen van klinische ziekte uitingen tussen patiënten enorm verschillend zijn.

In hoofdstuk drie exploreren we hoe Ussing metingen van het behandeleffect op gekweekte neuscellen van individuele patiënten correleren met klinische effecten bij deze patiënten. Aangezien de in vitro en in vivo effecten gecorreleerd zijn, zou het in de toekomst een nuttige manier kunnen zijn om behandeleffecten te kunnen voorspellen op basis van in vitro respons, als patiëntvriendelijke en haalbare aanvulling op rectale organoïden.

Hoofdstuk vier geeft een illustratie uit de praktijk van hoe verschillende methoden voor het meten van CFTR functie (FIS in rectale organoïden en Ussing in neuscellen) en follow up metingen zijn gebruikt voor een persoon met CF bij wie is vastgesteld dat zij in aanmerking kwam voor behandeling met ELE/TEZ/IVA op basis van metingen in organoïden, waarna zij een zeer goede klinische respons had op het middel.

In hoofdstuk vijf hebben we een groep F508del homozygote patiënten gevolgd die zijn gestart met LUM/IVA terwijl de FEV_1 90% of hoger was. Hoewel de longfunctie nauwelijks veranderde waren er wel veranderingen te zien in BMI, zweettest, exacerbatiefrequentie en kwaliteit van leven. Hieruit blijkt dat zich ook in deze subgroep van CF patiënten relevante effecten voordeden en dat het belangrijk is om op meer dan FEV_1 alleen te letten in de follow up van patiënten die met modulatoren starten, zeker bij patiënten met een ppFEV_1 die buiten de meestal onderzochte 40-90 valt.

Hoofdstuk zes biedt een extra inzicht in een ander follow up meting die gebruikt wordt bij CFTR modulatoren: de zweettest. We beschreven de daling in zweet chloride concentratie bij F508del homozygote patiënten na de start van LUM/IVA. De constateerden dat leeftijd en gewicht niet van invloed waren op de zweetchloride respons, maar geslacht wel. Het effect was bij vrouwen groter dan bij mannen. We

vonden in deze studie geen verklaring voor dit verschil en we vonden bij vrouwen geen betere effecten op FEV_1 of BMI.

Hoofdstuk zeven evalueert Brody scores die zijn berekend uit CT scans van F508del/S1251N patiënten voor de start van behandeling met ivacaftor, en de correlatie van deze score met het effect van behandeling op de longfunctie. Patiënten met een hogere Brody score hadden meer verbetering in de longfunctie van de behandeling. Dit suggereert dat in deze groep met relatief behouden longfunctie, de patiënten met de minste structurele schade aan de longen het minste ruimte voor verbetering hadden in de longfunctie.

Hoofdstuk acht beschrijft een groep CF patiënten met ernstige longschade die startten met ELE/TEZ/IVA in een compassionate use programma, en de behandeleffecten op korte en middellange termijn. Een indrukwekkend effect werd gezien op de CT scans van deze patiënten met vermindering van mucusplugging en peribronchiale verdikking, en ook werd een duidelijk effect op de FEV_1 en goede respons op BMI en exacerbatiefrequentie gezien. Overall hadden deze patiënten minder effect op de longfunctie, maar even goede andere uitkomsten in vergelijking met patiënten met matige longschade.

Hoofdstuk negen geeft een overzicht van verschillende relevante vragen die mensen met CF kunnen hebben wanner ze starten met effectieve CFTR modulatoren. De vragen zijn een voor een beantwoord met behulp van de op dat moment beschikbare evidence. Het artikel is bedoeld als handvat voor clinici wanneer zij het starten van de therapie met hun patiënt bespreken.

In de discussie vergelijken we de bevindingen in dit proefschrift met die uit de gepubliceerde literatuur, en worden verschillende kansen en noodzakelijkheden voor toekomstige onderzoeken benoemd.

List of abbreviations

ATP	Adenosine triphosphate
AUC	Area under the curve
BMI	Body mass index
cAMP	Cyclic adenosine monophosphate
CF	Cystic Fibrosis
CFQ	Cystic Fibrosis questionnaire
CFQ-R	Cystic Fibrosis questionnaire, revised
CFTR	Cystic Fibrosis Transmembrane conductance Regulator
CPET	Cardiopulmonary Exercise Testing
CT	Computed Tomography
ELE	Elexacaftor
ENaC	Epithelial sodium channel
FEV_1	Forced expiratory volume in one second
FIS	Forskolin induced swelling
IBMX	3-isobutyl-1-methylxanthine
ICM	Intestinal current measurement
IVA	Ivacaftor
LUM	Lumacaftor
MCID	Minimally clinically important difference
NPD	Nasal potential difference
PBS	Phosphate buffered saline
PD	Potential difference
$ppFEV_1$	Forced expiratory volume in one second, percentage of predicted
SCC	Sweat chloride concentration
SD	Standard deviation
SDS	Standard deviation score (Z-score)
SmPC	Summary of product characteristics
TEZ	Tezacaftor
TMEM16A	Transmembrane 16 channel, calcium activated
WT	Wildtype

Dankwoord

Een proefschrift ontstaat nooit door de inzet van maar één persoon. Het boekje dat voor u ligt ook zéker niet. Velen waren essentieel en nog meer mensen waren enorm behulpzaam bij het tot stand komen van ideeën, de praktische uitvoering van onderzoek, het aandragen van levenslessen en nog zoveel dingen meer.

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A2-lieverds, fijne nerdvrienden van me, van wie ik precies weet wat ik aan jullie heb, al sinds we met z'n allen op het Stedelijk Gym in de vensterbanken bivakkeerden in de pauzes (geen zorgen Valentijn, mijn flexibele geheugen plaatst jou daar moeiteloos tussen). Een groep waar iedereen volledig zichzelf kan zijn. Omdat we, alle verschillen ten spijt, al zo'n 15 jaar voldoende common ground vinden om het elke keer weer gezellig te hebben en elkaar door dik en dun te steunen waar dat nodig is. Dat is van onschatbare waarde. Ik hoop onze vakanties, WUE's en random ontmoetingsmomentjes dan ook nog lang mee te maken. Jullie zijn schatten.

Rigtje, ik ben elke dag opnieuw zo ontzettend blij met onze vriendschap. Jij bent er altijd wanneer het ertoe doet, en dat al sinds we een jaar of 7 waren. CF-inhoudelijk hebben we een soort symbiose ontwikkeld. Ik snap door jouw ervaringen moeiteloos wat CF patiënten zoal meemaken en belangrijker, dat de veerkracht zó groot is, dat die maar moeilijk voor mogelijk te houden is. Andersom laat jij je bijpraten over CF-nieuws en laat me wat uitgebreider uitleggen waarvoor in het ziekenhuis even wat weinig tijd was. En daarna stappen we moeiteloos over op hele andere vragen des levens, of lichtere onderwerpen, of op gewoon woordeloos genieten. Van het moment. Of van iets met veel kaneel erin. En dan voel ik me zo geweldig rijk. Ik ben trots dat je als paranimf het voltooien van mijn promotieonderzoek met me deelt. Dat voelt zo ontzettend passend.

Louise, Jan, familie is de basis. Door die basis blijf ik altijd stabiel overeind. Jullie hebben grotendeels geen idee gehad van wat ik inhoudelijk nou aan het doen was. Dat jullie me desondanks – dus volkomen onvoorwaardelijk – altijd blijven steunen is dan ook bijzonder en getuigt van groot vertrouwen. **Carlijn, Joep,** jullie betrokken vragen hadden unieke invalshoeken die zeer gewaardeerd zijn. **Truus, Frederiek, Michel,** het is heel duidelijk dat de warme uitstraling van Dries in de genen zit. Ook vind ik het vaak heerlijk hoe jullie smullen van verhalen over mijn werk in het ziekenhuis. Door te zien dat jullie trots zijn, ben ik het zelf ook een beetje meer. **Douwe**, blijf alsjeblieft altijd zo ontwapenend met je enthousiasme over een spel, een dansje of over alweer een muziekinstrument van je gekke oom en tante.

Mirte en Koen, mijn engeltjes van kinderen, dat jullie stille geboorte in hoofdzaak iets verdrietigs was is een misvatting. Want jullie brengen trots door te bestaan, en troost door altijd bij me te zijn. Jullie hebben liefde gebracht in een vorm die ik eerder niet kende. Mijn kracht versterkt en mijn wereld verrijkt. Jullie niet zien opgroeien blijft altijd een gemis. Maar jullie blijven levend in mijn hart, en dat zal daardoor voor altijd een betere plek zijn.

Jesse, jij liet me doorgaan toen ik de laatste delen aan dit proefschrift toevoegde, en je zorgde vanbinnen trappelend dat ik dat met meer plezier deed dan ik had kunnen voorzien. Jij heerlijke hartenopener met je gulle lach, je creatieve plannetjes en je pretoogjes. Ik kan niet in woorden vatten hoe groot mijn geluk is met jou. Je bent elke dag mijn lichtje.

Andries, mijn geweldige lieve man, af en toe trap je even voor mij op de rem omdat ik téveel tegelijk wil, en wel nu. Maar de rest van de tijd breng je zoveel ondersteuning, zoveel mede-enthousiasme, zoveel trots op mijn vak, dat ik zonder jou juist lang zo hard niet zou kunnen gaan. Omdat wij precies dat perfecte team zijn is de balans in orde, is ons huis een thuis en is keihard werken niet stressvol. Het maakt me warm van binnen om te merken dat als ik weer eens uitgebreid over mijn werk uitweid, uit jouw reacties blijkt dat je tot in de details hebt onthouden wat ik eerder vertelde. Je hebt er in het dagelijks leven als fietsenmaker/barista/saxofoonleraar niks aan, maar je weet precies wat er gemeten wordt in een Ussing kamer, wat een stopcodon is, en waarom je voor het ‘fixen’ van F508del eiwit meerdere stoffen tegelijk nodig hebt. En dat... is aandacht en liefde op z’n puurst. Mijn schat, ik hou van je.

There is meaning and purpose to all of it. Thank God.

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Curriculum Vitae

Bente (Benedikt Louise) Aalbers was born December 25th, 1989 in Nijmegen, the Netherlands.

After finishing grammar school at Stedelijk Gymnasium Nijmegen in 2007, she went on to study medicine in Utrecht. She participated in the faculty's honours programme during the master, which introduced her to CF research.

Directly after graduating her medical degree in 2013, she started as a resident in respiratory medicine, first in the department of internal medicine at Diakonessenhuis Utrecht, and from 2015 in the department of Pulmonology at UMC Utrecht, while working on research projects on the side.

In 2018 she was granted the opportunity to pause her residency to work on research full time for a period of 3 years. In between, due to the COVID pandemic in 2020-2021, she returned to the clinic for half a year before finishing the research and resuming her residency at UMC Utrecht in 2022.

In February 2024 she started working as a pulmonologist at Haga Hospital in The Hague, and since December 2024 she works at CWZ in Nijmegen in the field of pulmonary oncology.

Bente lives in Utrecht with her husband Andries. In 2021 they became parents of their daughter Mirte and son Koen, and in 2023 their son Jesse was born.

In her free time, Bente enjoys practicing sports (swimming, cycling, running) and music (singing, saxophone) as well as cooking or baking for her loved ones.