Clinical pharmacology of CFTR modulators

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(met een samenvatting in het Nederlands)

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General introduction

The landscape in cystic fibrosis (CF) care has changed dramatically over the past few years. Progress has been made in understanding the consequences of mutations in the cystic fibrosis transmembrane regulator (CFTR) gene, which gave rise to the development of new drugs. These CFTR modulating drugs are able to partially restore the functional expression of specific CFTR mutations and thereby facilitate the treatment of the underlying cause of the disease instead of treating symptoms.

The pathophysiology of cystic fibrosis, a focus on mutations in the cystic fibrosis conductance regulator gene

CF is an autosomal recessive hereditary disease caused by mutations in the CFTR gene, which result in impairment of CFTR mRNA and protein expression, function, stability or a combination of these. The CFTR protein is an epithelial ion channel that regulates chloride and bicarbonate transport throughout the body. Absent or dysfunctional protein leads to abnormal ion and water transport in multiple organs including airways, gastrointestinal tract, reproductive tract, and secretory glands [1-3]. This results in disruption in airway clearance of mucins and increases in bacterial colonization, pancreatic insufficiency, and intestinal obstruction [1-3]. Since the discovery of the CFTR gene in 1989, over 1700 mutations have been reported to cause CF [4]. Based on the functional defect in the CFTR protein these mutations can be divided into six different classes (table 1). These classes of CFTR mutations (genotype) are associated with different characteristics of CF disease (phenotype) depending on the residual function. Cystic fibrosis is a multi organ disease characterized by substantial clinical heterogeneity. Even among people with CF (pwCF) with the same CFTR genotype there is substantial clinical variation [5].

Table 1. *Classes of CFTR mutations, the primary defect and the outcome*

Mutation class	Defect	Outcome
I	Protein production	Complete absence of CFTR protein due to premature mRNA termination (nonsense or frame shift mutation)
II	Protein processing	Inability of protein to localize to correct cellular location due to abnormal post-translational modifications
III	Protein regulation	Decreased activity of protein (chloride channel) in response to ATP due to abnormalities of the nuclear binding fold regions
IV	Protein conduction	Frequency of flow of ions and channel opening duration are reduced though there is generation of chloride currents on stimulation with cAMP
V	Reduced amount of functional CFTR	Stability of mRNA and/or mature protein is compromised
VI	Normal amount of functional CFTR	Enhanced turnover due to C-terminus abnormalities

CFTR modulators

Until recently, the only available treatments for CF were directed to CF related complications. Although this treatments resulted in an inhibition in lungfunction decline and an improved survival, they failed to cure the disease. Treatment included mucolytic agents, inhaled and systemic antibiotics, anti-inflammatory drugs and pancreatic enzyme replacement therapy. In the last decade, enormous progress has been made with the development of the so-called CFTR modulators. To date, four CFTR modulators have been approved by the European Medicines Agency and US Food and Drug Administration for use in pwCF and specific mutations. These therapies show a life changing benefit in many pwCF. With the development of CFTR modulators, a promising era of targeted therapy has commenced. Current developments might prevent the complications of CF and further increase life expectancy of many pwCF. However, CFTR modulators are almost exclusively available in the world's richest countries. Around 162.500 people are estimated to be living with CF across 94 countries. Of these, an estimated 65% are diagnosed, and approximately 19.000 (12%) are receiving triple combination therapy (a higly effective CFTR modulator

combination). The extremely high costs of these drugs are a huge barrier to effective and equitable treatment.

Although CFTR modulators show a robust clinical effect at group level in pwCF with specific mutations, the individual effect is variable. In daily practice, more side effects of these drugs are seen than expected based on the registration studies. The interpatient variability of clinical benefit and prevalence of side effects stresses the need to better understand the pharmacology of these drugs.

In this thesis we studied aspects of the clinical pharmacology of these drugs. We mainly focussed on pharmacokinetic aspects, variability between pwCF with different disease phenotypes and drug-drug interactions (DDI's). Aspects needed to understand the clinical pharmacology of CFTR modulators are explained in the next paragraphs.

What CFTR modulators do to the body, "pharmacodynamics"

CFTR modulators improve CFTR function either through potentiation of the abnormal protein channel at the cell surface (ivacaftor) [6-8], or through correction of protein transport to the cell surface (lumacaftor, tezacaftor, elexacaftor) (figure 1.) [9-14].

CFTR potentiators

Ivacaftor (VX-770, Kalydeco®), currently the only approved CFTR potentiator, facilitates increased chloride transport by potentiating the channel-open probability (or gating) of the CFTR protein at the cell surface [15, 16]. The exact mechanism of how this potentiation works is unknown, though it may be through decoupling the gating cycle and ATP hydrolysis cycle, or by increasing the ATP-dependent opening rate and slowing the closing rate [17, 18]. It is believed that by binding to CFTR in the epithelial cell membrane, ivacaftor improves the function of both CFTR with gating mutations and CFTR with normal function [17,19]. Clinical trials in which patients were treated with ivacaftor showed an impressive improvement in clinical outcome measures as pulmonary function measured by the Forced Expiratory Volume in 1 second (FEV_1) by 10,4 – 17,5% [15, 20]. Ivacaftor is registered as monotherapy for different class III and IV mutations of the CFTR gene, and in combination with CFTR correctors.

CFTR correctors

CFTR correctors increase the amount of CFTR at the cell surface by improving the trafficking of mutant CFTR to the cell surface [9-11]. Currently, three correctors are registered: Lumacaftor is registered in combination with ivacaftor (Orkambi®) for patients homozygous for F508del mutation. Lumacaftor is a first generation CFTR corrector and acts directly on F508del-CFTR to improve its cellular processing and trafficking, thereby increasing the quantity of functional CFTR at the cell surface [9]. Tezacaftor is registered in combination with ivacaftor (Symkevi®) for patients homozygous for F508del mutation or F508del mutation and a specific residual function mutation. Tezacaftor is also registered in combination with ivacaftor and elexacaftor (Kaftrio®) for patients with at least one F508del mutation. Tezacaftor binds to the first Membrane Spanning Domain (MSD-1) of CFTR and has the same mechanism of action as lumacaftor [11, 12]. Elexacaftor is registered in combination with tezacaftor and ivacaftor (Kaftrio®). Elexacaftor binds to different sites on the CFTR protein, leading to an additive effect in facilitating the cellular processing and trafficking of F508del-CFTR and thereby increasing the amount of CFTR protein delivered to the cell surface. Clinical trials showed an impressive effect of triple therapy with ETI (elexacaftor/tezacaftor/ivacaftor) in pwCF with at least one F508del and a minimal function (MF) mutation and in pwCF who are homozygous for the F508 del mutation. FEV_1 (percent predicted) increased with 13,8 and 13,5 percentage points respectively compared to placebo [13,14]. Recently, the FDA approved ETI in pwCF of 12 years and older with at least one of 177 newly approved-mutations other than F508del. The results are promising and show the potential of life changing improvements for many patients.

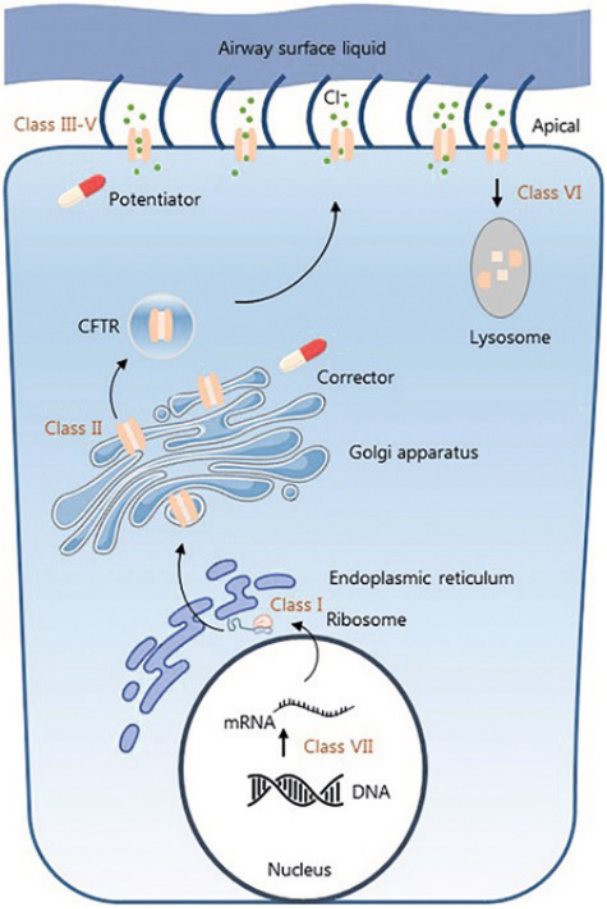

Figure 1. *The CFTR pathway and mechanism of action of CFTR modulators, adapted from a previously published figure [21].*

What the body does to CFTR modulators, “pharmacokinetics”

Real-world-clinical experience revealed a higher number of side effects of CFTR modulator treatment than was expected based on data from clinical trials. Severe side effects as liver injury, severe rash or psychological changes sometimes lead to the decision to stop treatment with CFTR modulators which often causes deterioration of the disease. Also, a high interpatient variability in clinical benefit has been observed which has already been reported in clinical trials with a change in $ppFEV_1$ ranging from -2,5% to > +20% [13, suppl. data]. These observations raise questions about the relationship between dose and response to these drugs. The treatment effect in an individual patient is a result of pharmacodynamics and pharmacokinetics. The diversity in characteristics of the disease within the CF population may influence pharmacokinetics and thereby result in variability in treatment (side) effect.

Absorption, distribution, metabolism, and excretion (“ADME”) are processes that together describe the disposition of a drug (figure 2) and explain how pharmacokinetic processes happen. Absorption is defined as how the drug moves from the site of administration to the blood; distribution is the movement of a drug from the systemic circulation to the tissues; metabolism is the conversion of a drug to metabolites that can be eliminated from the body, and excretion is the irreversible loss of a substance from the system. Characterization of these “ADME” properties helps to understand variability in drug exposure between individuals.

It is already known that certain drugs have altered pharmacokinetic properties in pwCF compared to non CF subjects [22,23]. With the development of highly effective modulators and variability in treatment effect, the CF population may even become more heterogenic and differences in pharmacokinetic properties within the CF population itself (interpatient variability) may become more important. Also, pharmacokinetic properties of certain drugs may alter after restoring CFTR function in pwCF who are starting treatment with highly effective modulators, which may increase intrapatient variability.

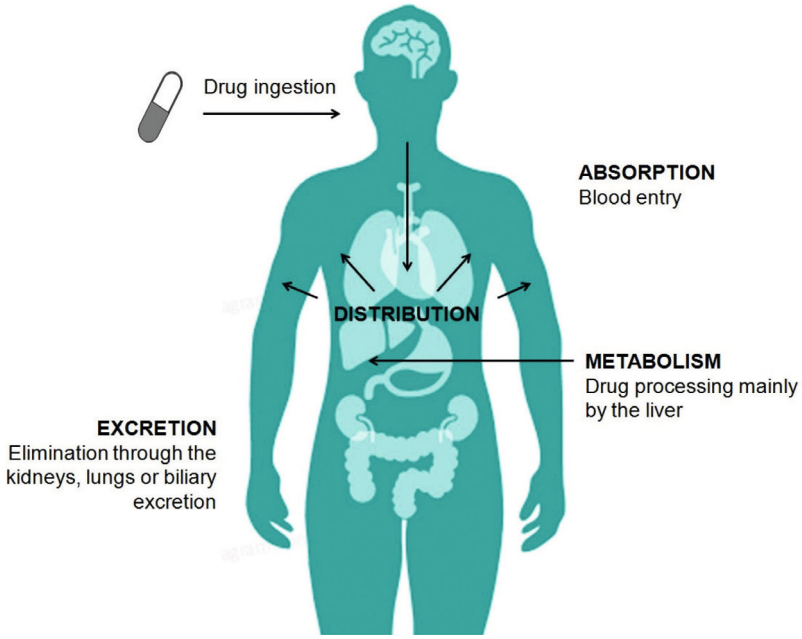

Figure 2. *ADME principles.*

Aim and outline of this thesis

In this thesis we aim to improve insight in the clinical pharmacology of CFTR modulators with the main focus on pharmacokinetics and features of CF disease that may contribute to variation in drug exposure. In **chapter 2** we review data on exposure response relationship of CFTR modulators with a special focus on pharmacokinetic features of CF disease that may affect drug exposure. Also we discuss situations that may give reason for reconsideration of dosing regimens.

Co-administration of CYP3A4 inhibitors will affect the pharmacokinetics of ivacaftor. Advices for dose adjustment are based on studies in healthy subjects. In order to provide a well-founded dosing advice we investigate the pharmacokinetic interactions between ivacaftor and cytochrome P450 3A4 inhibitors in pwCF and healthy controls in **chapter 3**.

In **chapter 4** we evaluate if the exocrine pancreatic function changes the degree and rate of absorption of ivacaftor and thereby changes drug exposure.

PwCF after solid organ transplantation are in general excluded from clinical and registration studies because of expected DDI's. Since highly effective CFTR modulators are expected to have the same impressive effect in pwCF after solid organ transplantation other than lung, we investigate the use of ETI in liver or kidney transplanted pwCF using tacrolimus with a focus on DDI in **chapter 5**.

In **chapter 6** we focus on pharmacodynamics by investigating the clinical effect of lumacaftor/ivacaftor in people with cystic fibrosis with an A455E–CFTR mutation (a rare CFTR mutation with a prevalence of 4,1% in the Netherlands) and the correlation with organoid-based measurements.

A real life example showing the importance of improving our knowledge of pharmacokinetic features of CFTR modulators and its relationship with drug exposure is presented in a case report in **chapter 7**.

Despite the promising developments of targeted therapy with CFTR modulators, treatment of this complex multi-organ disease still comprises a high amount of other drugs. In **chapter 8** we will give an overview of most important studies about pharmacotherapy in CF focussing on side effects, DDI's and options to deal with drug induced toxicity.

Finally in **chapter 9** we discuss our results and the current status regarding to the use of therapeutic drug monitoring of CFTR modulators.

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2

CFTR modulators: Does one dose fit all?

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Abstract

For many people with cystic fibrosis (pwCF), cystic fibrosis transmembrane regulator (CFTR) modulators will be the cornerstone of their treatment. These modulators show robust treatment effects at group level in pwCF with specific mutations. The individual effect however, is variable. In this review we will explain reasons for reconsideration of dosing regimens of CFTR modulating therapy in order to improve treatment response and prevent side effects. Since the effect of a drug depends on pharmacodynamics (PD) and pharmacokinetics (PK), PD and PK properties of CFTR modulators will be discussed. PK-PD relationships will be used to gain insight in dosage response and exposure response relationships. To understand the cause of variation in drug exposure, pharmacokinetic properties that may change due to CF disease will be explained. We show that with current insight, there are conceivable situations that give reason for reconsideration of dosing regimens, however many questions need to be unravelled.

Introduction

Cystic fibrosis (CF) is a chronic, hereditary, multi-organ disease caused by absence or dysfunction of the cystic fibrosis transmembrane conductance regulator (CFTR) protein [1]. Over the past decade, CFTR protein modulators have been developed, which improve CFTR function either through potentiation of the abnormal protein channel at the cell surface (ivacaftor), or through correction of protein transport to the cell surface (lumacaftor, tezacaftor, elexacaftor). These treatments have now been approved by the European Medicines Agency and US Food and Drug Administration for use in people with CF (pwCF) and specific mutations. With the development of the CFTR modulators, a new era in CF treatment has arrived. Recent trials show an impressive clinical effect of combination therapy with elexacaftor plus tezacaftor plus ivacaftor. Heijerman et al. showed an increase in forced expiratory volume in one second (FEV_1) of 10 percentage points in patients homozygous for the F508 del mutation ([95% CI 7.4 to 12.6], p<0.0001) after 4 weeks of treatment with elexacaftor/tezacaftor/ivacaftor compared to tezacaftor/ivacaftor [2]. Also, elexacaftor/tezacaftor/ ivacaftor was shown to be efficacious in pwCF with F508del-minimal function genotypes, in whom previous CFTR modulator regimens were ineffective. For this genotype, Middleton et al. showed a 13.8 points higher $ppFEV_1$ at 4 weeks and 13.4 points through week 24 compared to placebo [3]. These results are promising and show the potential of life changing improvements for these patients.

For many pwCF, CFTR modulators will be the cornerstone of their treatment. Although these modulators show robust treatment effects at group level, the individual effect is variable [4, 5]. The effect of a drug in an individual patient is a result of what the drug does to the body (pharmacodynamics) and what the body does to the drug (pharmacokinetics). We will briefly mention pharmacodynamic properties of CFTR modulators but an extensive explanation of pharmacodynamics is out of the scope of this review. In order to gain insight in the degree to which the drug dosage influences the treatment effect, we will review data on exposure response relationship of these drugs first. Second, we will focus on pharmacokinetic principles and features of CF disease that may contribute to variation in drug exposure.

Registered dosing recommendations of CFTR modulators are based on pharmacodynamic effects in vitro, serum pharmacokinetic studies, and early dose escalating (phase II) studies. In the final part of this review we will discuss remaining questions which need to be resolved in order to determine if current dosing strategies can be applied to all patients or need reconsideration in certain patient groups.

Pharmacodynamics of CFTR modulators

Mutations in the CFTR gene lead to dysfunction of the CFTR ion channel [6]. A group of drugs named CFTR modulators, have been developed to improve this function. Ivacaftor, currently the only approved CFTR potentiator, facilitates increased chloride transport by potentiating the channel-open probability (or gating) of the CFTR protein at the cell surface [7]. Currently three correctors entered the market: lumacaftor, a first generation CFTR corrector, acts directly on F508del-CFTR to improve its cellular processing and trafficking, thereby increasing the quantity of functional CFTR at the cell surface [4]. Tezacaftor, a second generation CFTR corrector that binds to the first membrane spanning domain (msd-1) of CFTR and has the same mechanism of action as lumacaftor [5, 8]. Most recently, elexacaftor, a next generation corrector that binds to different sites on the CFTR protein than tezacaftor, leading to an additive effect in facilitating the cellular processing and trafficking of F508del-CFTR and thereby increasing the amount of CFTR protein delivered to the cell surface.

The exact mechanisms by which lumacaftor, tezacaftor and elexacaftor improve cellular processing and trafficking of F508del-CFTR, and ivacaftor potentiates F508del-CFTR are not known.

Ivacaftor is registered as monotherapy for specific gating mutations in the CFTR gene, or in combination with lumacaftor (for patients homozygous for F508 del mutation), in combination with tezacaftor (for patients homozygous for F508del mutation or F508del mutation and specific residual function mutations), and in triple combination with both elexacaftor and tezacaftor (for patients with at least one F508del mutation) [9].

Dose response relationships of currently registered CFTR modulators were investigated in phase II studies in adult pwCF with specific genotypes. Pharmacodynamic endpoints, e.g. FEV_1, nasal potential difference (NPD), and sweat choride were measured and compared between study groups with escalating dosing regimens. For ivacaftor, lumacaftor and tezacaftor monotherapy, a trend of increasing response with higher dose was observed [10-12]. A range of doses for ivacaftor in combination therapy was not studied. For elexacaftor combined with tezacaftor and ivacaftor, no clear dose response has been seen, as the 100 mg arm showed a response lower than the 50 mg and 200 mg arm. These results could suggest a rather flat dose-response curve or a maximum effect at a dose level below the tested dosages [13].

Exposure response relationship

Dosing regimens of approved CFTR modulators are based on pharmacodynamic effects in vitro (data not published), serum pharmacokinetic studies, and early dose ranging (phase II) studies. Robust treatment effects of CFTR modulating therapies in pwCF with specific genotypes have been demonstrated. However, high variabilitiy in treatment response has been observed in individual patients with the same genotype and treatment dosage. The question is whether the same dosage of CFTR modulators results in differences in drug exposure and thereby variation in treatment response. In this chapter we will focus on the importance of understanding the exposure response relationship of CFTR modulators, we will discuss what is currently known, and propose methods to investigate exposure response relationships.

Importance of understanding exposure response relationships

Among various pwCF, high variability in clinical response to CFTR modulating therapy has been observed [4, 5, 8, 14]. The underlying causes of different drug responses and clinical outcomes might be partially attributed to variation in drug exposure. In this context, knowledge of the therapeutic window is important. The therapeutic window (or pharmaceutical window) of a drug is the range of drug concentrations which can treat disease effectively without having toxic effects. In clinical trials, CFTR modulators were generally well-tolerated, with the exception of lumacaftor/ivacaftor which showed a higher rate of respiratory-related adverse events [4]. Observational studies with real-world CFTR modulator safety data however, have shown higher rates of discontinuation as well as adverse events that were rarely observed and not described in the clinical trial setting [15]. Regarding the therapeutic window of ivacaftor it is important to mention data from several studies in target tissues reporting destabilization of corrected F508del CFTR by too high ivacaftor concentrations, dramatically increasing its turnover rate. Chronic ivacaftor treatment also reduced mature wild-type CFTR levels and function [16-18]. This suggests that a too high ivacaftor exposure can do harm. This underlines the importance of knowledge of the exposure effect relationship of this drug. These findings also demonstrate that chronic treatment with CFTR potentiators and correctors may have unexpected effects and may require optimization of dosing regimens.

Exposure response relationship of CFTR modulators, what do we know?

The results of an exposure-response analysis for ivacaftor can be found in the FDA report [10]. Phase II studies showed no additive effect of ivacaftor dosage 250mg q12h over 150mg q12h. A direct maximal effect (E_{max}) model which was used to define the relationship of FEV_1 and sweat chloride with ivacaftor exposure in pwCF. Ivacaftor

dose of 150 mg q12h was selected based on simulations showing that this dose would be needed to achieve an average steady state ivacaftor trough concentration (C_{min},ss) of at least the estimated concentration at which the effect is at 90% of the maximum (EC90) for FEV_1 endpoint and 84% (EC84) value for sweat chloride endpoint. This trough concentration was estimated to be approximately 0.25 µg/mL. As shown in table 1 mean (SD) Cmin of ivacaftor is above this level, 0.8 (0.3) µg/mL. Because no specific dose-limiting safety concerns were identified in early dose escalation studies no exposure-response analysis for safety was performed. However, in daily practice side effects in patients on ivacaftor treatment have been observed, which arises questions about the potential concerns of too high ivacaftor exposure [15].

Data in the FDA report [11] for lumacaftor/ivacaftor show a greater reduction in sweat chloride with increasing lumacaftor concentrations and a slight increase in effect with the addition of ivacaftor. The concentration at which the effect is at 50% of the maximum (EC50) of lumacaftor for sweat chloride was estimated at trough levels of 4.5 µg/ml. For tezacaftor the average EC50 was 0.5 µg/mL for sweat chloride and 0.4 µg/mL for $ppFEV_1$. No data about target levels of ivacaftor, other than the slightly increase of Emax (for sweat chloride and $ppFEV_1$) by adding ivacaftor to tezacaftor, are mentioned [12]. An In vitro study in F508del/F508del and F508del/MF human bronchial epithelial cells, show that elexacaftor concentration-dependently enhances chloride transport with a larger effect than achieved by tezacaftor/ivacaftor. EC50 for elexacaftor in combination with tezacaftor/ivacaftor has been estimated in vitro but no in vivo data are available [13].

Due to development of new CFTR modulators, many pwCF have changed their CFTR modulator regimen. For CF patients homozygous for the F508 mutation currently three CFTR modulator regimens are approved by the FDA and EMA. Many patients have now switched from lumacaftor/ivacaftor to tezacaftor/ivacaftor or more recently, to elexacaftor/tezacaftor/ivacaftor. In several patients we observed differences in clinical outcome and tolerability after changing lumacaftor/ivacaftor to tezacaftor/ivacaftor. This arises questions about changes in drug exposure when switching from one modulator regimen to another. We have measured steady state trough levels of lumacaftor and ivacaftor in 24 adult CF patients who planned to switch to tezacaftor/ivacaftor and we measured trough levels of tezacaftor and ivacaftor in steady state in the same patients. Although tezacaftor/ivacaftor treatment contains a lower dose of ivacaftor compared to the lumacaftor/ivacaftor (300 vs 500 mg/day), ivacaftor trough concentrations were 7 times higher after tezacaftor/ivacaftor treatment compared to lumacaftor/ivacaftor treatment (mean 7.08, range 1.12-34.30; p=0.00 (Wilcoxon),

unpublished data). If this increased exposure to ivacaftor observed within individual patients is clinically relevant needs to be elucidated.

How to obtain insight in exposure response relationship of CFTR modulators?

Plasma and cellular drug concentrations

For drugs such as CFTR modulators that act within cells, intra cellular concentrations would ideally be obtained to be related to treatment effect. Peripheral blood however, is easily accessible and would allow to monitor the pharmacokinetic profile of CFTR modulator treatment at patient level. Analytical methods have been developed and validated for rapid detection and quantification of ivacaftor, its major metabolites, lumacaftor and tezacaftor in the plasma and sputum of pwCF [19, 20]. Guimbellot et al. observed a correlation between plasma and cellular ivacaftor concentrations, but cellular concentrations were disproportionally more elevated in patients with higher plasma concentrations [21]. This suggests in vivo accumulation of ivacaftor, which has also been mentioned in in vitro reports [22]. The higher cellular concentrations may result in a level of CFTR restoration distinct from what would be expected from plasma concentrations.

Organoids

Plasma samples from CFTR modulator-treated CF patients have been used to personalize pharmacokinetics and pharmacodynamics by organoid testing. The primary readout (forskolin-induced swelling or FIS) is CFTR dependent, and there is evidence for a correlation between the modulator-induced FIS response and the change in FEV_1 and sweat chloride concentration in vivo [23]. Dekkers et al. described a bioassay to measure CFTR modulator activity in human plasma using intestinal organoids. They observed a dose-dependent increase of forskolin-induced organoid swelling for ivacaftor [24]. Organoid assays may help us to better understand the relationship between drug exposure and treatment response.

Pharmacokinetics of CFTR modulators and CF features that may change pharmacokinetic properties

Pharmacokinetics shows what the body does to the drug. Different features of CF disease may influence pharmacokinetic properties of drugs which may contribute to variation in drug exposure. In order to gain insight in the pharmacokinetics of a certain drug, we will explain four main pharmacokinetic processes: absorption, distribution, metabolism and excretion [25] with a focus on CFTR modulators.

It is already known that certain drugs have altered pharmacokinetic properties in pwCF compared to non CF subjects [26, 27]. Although little is known about the intersubject variability in the CF population, some studies have been published showing differences between CF patients in clinical pharmacokinetic parameters of several drugs [28-30]. With the development of highly effective modulators, the CF population may even become more heterogenic and differences in pharmacokinetic properties within the CF population itself (interpatient variability) may become more important. Also, pharmacokinetic properties of certain drugs may alter after restoring CFTR function in pwCF who are starting treatment with highly effective modulators, which may increase intrapatient variability. In this chapter we will discuss features of CF disease that may change pharmacokinetic properties and thereby may cause inter- and intrapatient variability in drug exposure.

Oral absorption

Basic principles

Absorption is defined as how the drug moves from the site of administration to the prehepatic bloodstream. Absorption and the consequent first hepatic passage defines bioavailability (the fraction of the drug that reaches the systemic circulation). Orally administered medication may have variable bioavailability which depends on several factors including the disintegration and dissolution of solids, gastric emptying rate, dietary content, first hepatic pass effect, presence of interacting medication and the acidity of gastric contents.

Oral absorption of CFTR modulators

Administered as an oral dose, CFTR modulators are absorbed directly from the gut. All CFTR modulators should be taken with fat containing food because the bioavailability of ivacaftor, lumacaftor and elexacaftor increases by 2 to 4 times compared to a fasting

state. The exposure of tezacaftor does not change when given with a fat meal (SmPC tezacaftor). Data of maximal concentration (Cmax) and time to maximal concentration (Tmax) for all registered CFTR modulators are shown in table 1.

CF characteristics that affect absorption

Drug absorption in patients with CF can be affected by alterations in several factors which we will discuss here [31, 32].

Gastro and intestinal transit time: CFTR dysfunction in the intestines causes a decreased water secretion resulting in thick viscous intestinal content with a high risk of intestinal obstruction and delayed transit [32, 33]. Also gastroparesis is a common problem seen in CF patients, especially in patients with poorly controlled cystic fibrosis related diabetes (CFRD) [34]. This delayed motility may contribute to a decreased absorption rate for certain drugs.

Pancreatic insufficiency: A severe CFTR gene mutation in both alleles results in little or no CFTR chloride channel activity and destruction of the exocrine pancreas [35]. This exocrine pancreatic enzyme deficiency impairs the absorption of dietary fats and lipid-soluble nutrients [36]. Around 85% of pwCF develop exocrine pancreatic insufficiency and despite treatment with pancreatic enzymes patients still suffer from fat malabsorption [37]. This may result in a decreased and delayed absorption of oral drugs. The influence of exocrine pancreatic insufficiency and the effect of treatment with pancreatic enzymes was studied by Dickinson et al. They showed that although pancreatic enzyme replacement improved the absorption characteristics of the chloramphenicol-P formulation, absorption remained prolonged and unreliable. They also showed that exocrine pancreatic insufficiency causes a decreased exposure to drugs that need pancreatic enzymes for the liberation of their active form, e.g. chloramphenicol [38].

Increased bile acid excretion and duodenal hyperacidity: Another gastro intestinal complication in CF is an increased fecal bile acid (BA) excretion [39, 40]. In the physiological situation the enterohepatic circulation of BAs is a tightly regulated system in which around 95% of total BAs is reabsorbed and the remaining 5% is excreted via the feces. BAs are important for digestion and absorption of fat and fat soluble vitamins. Theoretically this BA dysfunction may play a role in decreasing the exposure to lipophilic drugs in CF patients. CFTR dysfunction is related to postprandial hyperacidity of the duodenum which is caused by increased gastric acid secretion and decreased bicarbonate secretions in the intestine [41, 42]. This acidic environment may decrease drug absorption in CF patients.

When treatment with CFTR modulators is started in an early stage of the disease, organ function may improve. CF patients with pancreatic insufficiency may become pancreas sufficient and thereby drug absorption and exposure may increase. [43, 44].

Distribution

Basic principles

Distribution is the movement of a drug from the systemic circulation to the tissues. Distribution occurs most rapidly into body compartments with a high blood flow (lung, liver, brain). If the volume of distribution which is calculated from plasma concentrations is larger than the body volume, accumulation in plasma cells or tissues occurs. Major factors affecting distribution of drugs are diffusion rate, affinity of the drug to the tissue, perfusion, and binding to plasma proteins. This plasma protein binding (often to albumin) is often reversible and can act as a reservoir. High plasma protein binding results in a lower volume of distribution (Vd) (the amount of drug administered divided by the plasma concentration of that drug). For drugs with a high extravascular binding or storage in fat or other tissues, the volume of distribution is high.

Distribution of CFTR modulators

All CFTR modulators are transported in the plasma highly bound (99%) to plasma proteins to their site of action, which is the apical membrane of epithelial cells [45-48]. Volume of distribution for all registered CFTR modulators are shown in table 1.

CF characteristics that affect distribution

CF patients are at risk for malnutrition due to malabsorption, increased energy expenditure and a reduced food intake. Because many pwCF weigh less than healthy subjects but have a relatively higher lean body mass/fat free mass, the extracellular volume of an underweight CF patient will be underestimated when only total bodyweight is taken into account [26]. A higher volume of distribution in CF patients for some drugs can still be found after correction for body composition [49]. This may be caused by an increased total body blood volume and hypoalbuminemia which theoretically may lead to decreased protein binding. This hypoalbuminemia is associated with liver disease, cachexia and inflammation, problems often seen in CF patients [50].

With the introduction of highly effective modulators we expect differences in body composition between and within patients to become more prevalent [51].

Metabolism

Basic principles

The goal of metabolization is to make the drug easier to excrete. The enzymes involved in metabolism are present in many tissues but mainly in the liver [25]. It involves enzymes that convert prodrugs to active metabolites or convert active drugs to inactive or excretable forms. The liver's primary mechanism for metabolizing drugs is via a specific group of cytochrome P-450 enzymes, a microsomal superfamily of isoenzymes that catalyzes oxidation and hydroxylation of many drugs. CYP450 enzymes can be induced or inhibited by many drugs and substances.

Drug metabolism rates vary among patients and are influenced by genetic factors, coexisting disorders (particularly chronic liver disorders and advanced heart failure), and drug interactions (especially those involving induction or inhibition of metabolism).

Metabolism of CFTR modulators

Ivacaftor, tezacaftor and elexacaftor are extensively metabolized in the liver mainly by cytochrome P450 3A (CYP3A), including both CYP3A4 and CYP3A5. Lumacaftor however, is not extensively metabolized in humans and the majority of lumacaftor is excreted unchanged in the feces. M1 and M6 are the two major metabolites of ivacaftor in humans. M1 is considered pharmacologically active [45]. Administered together with lumacaftor, the steady-state exposure of ivacaftor is decreased due to the CYP3A inducing effect of lumacaftor [46]. M1-TEZ, M2-TEZ, and M5-TEZ are the three major circulating metabolites of tezacaftor in humans. M1-TEZ has similar potency to that of tezacaftor and is considered pharmacologically active, M2-TEZ is much less pharmacologically active and M5-TEZ is not considered pharmacologically active [47]. M23-ELX is elexacaftors only major circulating metabolite and is considered pharmacologically active with similar potency to elexacaftor [48].

CF characteristics that affect metabolism

The capacity of the liver to metabolize drugs depends on hepatic blood flow and liver enzyme activity. Factors that may change hepatic metabolism depend on the kind of drug. Drugs with a low hepatic extraction ratio are not sensitive to liver blood flow changes. The fraction of these drugs removed from the blood during a single passage through the liver (the extraction ratio) is small, so their clearance mainly depends on the activity of drug metabolizing enzymes.

Hepatic metabolism in pwCF may differ due to altered liver enzyme activity and or/ changes in liver blood flow.

Changes in liver enzyme activity: Enhanced hepatic metabolism in CF patients compared to healthy people has been described and may be caused by selective up-regulation of certain enzymes (e.g. cytochrome P450) [26]. However, more recent studies show the opposite, with non-altered CYP1A2, CYP2D6, xanthine oxidase and N-acetyltransferase activities and no increase of CYP3A4 expression in the gut in children with CF [52, 53]. Others investigated the association of infection and inflammation, which are common characteristics of CF disease, with a lower expression and activity of hepatic drug-metabolising enzymes (e.g. CYPs) [54, 55].

With increased life expectancy, which is in part due to better treatment options, the burden of pharmacotherapy in CF patients will increase, resulting in a higher risk for drug-drug interactions. An example of a common drug interaction is co-treatment of a CYP3A4 inhibitor (e.g. azoles) with a CYP3A4 substrate and inhibitor (e.g. cyclosporine) [56]. These interactions complicate the interpretation of hepatic metabolism and its influence on expected drug exposure. Therefore, for several drugs (e.g. azoles, immunosuppressants), therapeutic drug monitoring is currently advised.

Changes in liver blood flow: Liver disease in CF is a common problem [57] and can alter the kinetics of certain drugs [58]. Liver blood flow can be reduced because of pathological alterations caused by liver disease, as in cirrhosis. There can be spontaneous porta-caval shunts. For drugs with a high first pass effect, the shunt may result in the drug bypassing the liver and reaching the systemic circulation directly. This results in increased systemic availability of the drug.

As already mentioned, CFTR modulators are substrates of CYP3A4 and CYP3A5. Due to interaction with CYP3A4/5 inhibitors or inductors, co-administration is not recommended or require dose adjustment as is incorporated in the SmPC's of CFTR modulators. As therapeutic target ranges of CFTR modulators are currently unclear, therapeutic drug monitoring is not (yet) feasible in clinical practice.

Elimination

Basic principles

The kidneys are the principal organs for excreting water-soluble substances. The biliary system contributes to excretion to the degree that the drug is not reabsorbed from the gastrointestinal tract. Important principles in understanding elimination are clearance (the rate of elimination of the drug from the body and is the product of the

elimination rate constant and the volume of distribution) and half clearance time (the time required for the amount of drug present to be reduced by 50%).

Elimination of CFTR modulators

Following oral administration, the majority of ivacaftor, tezacaftor and elexacaftor is excreted in the feces after metabolic conversion (88, 72, 87% respectively). For lumacaftor, the majority (51%) is excreted unchanged in the feces. For ivacaftor, lumacaftor and elexacaftor urinary excretion is negligible, whereas 14% of tezacaftor is excreted in the urine. T1/2 and clearance values for all registered CFTR modulators are presented in table 1.

CF characteristics that affect elimination

In patients with CF, enhanced renal clearance has been observed for some drugs. In contrast to renal and hepatic clearance, biliary excretion might be decreased in CF [59]. Biliary disorders, prevalent in the CF population, could explain this phenomenon, but more research is needed to confirm this hypothesis.

CFTR modulators are mainly eliminated via feces and not with urine (tezacaftor only, 14%). Impairment of renal function is therefore not likely to change the elimination of CFTR modulators.

All four pharmacokinetic mechanisms will affect the exposure to the drug. The exposure (AUC) of the approved CFTR modulators in steady state, is shown in table 1.

Table 1. *Steady state pharmacokinetic parameters for CFTR modulators in a fed state in patients with CF aged 12 years and older. (data from SmPCs and FDA reports)*

	Cmax mean (SD) µg/mL	Tmax median (range) hours	Vd mean (SD) liters	T1/2 hours (SD)	Clearance mean (SD) L/h	AUC mean (SD) µg•h/mL	Cmin mean (SD) µg/mL)
Iva 150 mg q12h*	1.5 (0.6) ng/mL	4 (1-6)	353 (122) #	14.4 (3.9)	17.3 (8.4)	12.9 (3.6)	0.8 (0.5)
Lum 400mg q12h Iva 250 mg q12h	25.0(7.7) 0.6 (0.3)	4 (2-9) 4 (2-6)	86.0 (69.8) 201	25 (9.9) 9 (3.8)	2.4 25.1	198 (64.8) 3.7 (2.3)	9.8 (4.8) 0.08 (0.02)
Tez 100mg q24h Iva 150mg q12h	6.5 (1.8) 1.3 (0.4)	4 (2-6) 6 (3-10)	271 (157) 206 (82.9)	156 9	1.3 (0.4) 15.7 (6.4)	82.7 (23.3) 10.9 (3.9)	1.6 0.7
Elex 200mg q24h Tez 100mg q24h Iva 150mg q 12h	9.2 (2.1) 7.7(1.7) 1.2 (0.3)	6 (4-12) 3 (2-4) 4 (3-6)	53.7 (17.7) 82.0 (22.3) 293 (89.8)	25 60 13	1.2 (0.3) 0.8 (0.1) 10.2 (3.1)	162 (47.5) 89.3 23.2) 11.7 (4.0)	5.5 (2.7) 2.1(0.8) 0.8 (0.3)

Iva = ivacaftor, Lum = lumacaftor, Tez= tezacaftor, Elex= elexacaftor. SD: Standard Deviation (SD for clearance of lum/iva and Vd of iva as lum/iva combination and Cmin of Tez/iva were not shown in FDA reports); Cmax: maximum observed concentration; Tmax: time to maximum observed concentration; Vd: volume of distribution; T1/2: terminal half-life; AUC: area under the concentration versus time curve.

**ivacaftor steady state data presented show the average of study results in healthy volunteers shown in the FDA report (steady state data for pwCF and healthy volunteers are comparable as mentioned in the FDA report, reference ID: 3073639). # data after single dose.*

Conclusion and future perspectives

At group level CFTR modulators have shown robust treatment effects in pwCF with specific mutations. Results of both ivacaftor and elexacaftor/tezacaftor/ivacaftor therapy are impressive [7, 2, 3]. However, treatment effects differ between individual CF patients with similar genotypes. In this review we wanted to give insight in reasons for reconsideration of dosing regimens of CFTR modulating therapy in order to improve treatment response and prevent side effects. Knowledge about pharmacodynamics and pharmacokinetics and finally PK-PD relationships of CFTR modulators is therefore needed.

For ivacaftor, lumacaftor and tezacaftor a trend of increasing treatment response with higher dose was observed in phase II and III studies [10-12]. To evaluate if differences in treatment effects between patients treated with the same dosage may be caused by difference in exposure, knowledge about the therapeutic window of these drugs is needed. Exposure to ivacaftor was linearly correlated with response, and maximal effect concentration for FEV_1 (EC90) and sweat chloride (EC84) was 0.25 µg/mL [10]. The maximal effect concentrations (EC50) of lumacaftor and tezacaftor were estimated at trough levels of 4.5 and 0.5 µg/ml respectively. Maximal effect concentrations of ivacaftor as part of combination therapy have not been investigated.

In table 1 we summarized pharmacokinetic parameters from different CFTR modulators. C_{min},ss of ivacaftor, lumacaftor and tezacaftor is higher than the estimated maximal effect concentrations. Remarkably, C_{min},ss of ivacaftor as part of lumacaftor/ivacaftor is a factor 10 lower than C_{min},ss of ivacaftor as part of tezacaftor/ivacaftor treatment and ivacaftor monotherapy (table 1). The sponsor suggested that ivacaftor potency is 7-fold higher in F508del-CFTR (EC90 at 0.06 µg/ml) compared to G551D-CFTR (EC90 at 0.4 µg/ml) in the in vitro studies. However, this does not explain the difference with tezacaftor/ivacaftor since both are registered for F508 del homozygous mutations [60]. This arises questions about the ivacaftor dosage as part of combination therapy with CFTR correctors.

Features of CF disease which may change pharmacokinetic properties and thereby may affect drug exposure were explained. The influence of several conditions such as renal or hepatic impairment, body weight, and drug-drug interactions on drug exposure have already been investigated. Recommended dose adjustments can be found in the SmPC's of different CFTR modulators. Currently we are performing a study to investigate the influence of pancreatic function on the absorption and exposure

to ivacaftor in CF patients. The influence of other patient characteristics e.g. body composition on the exposure to CFTR modulators needs further investigation.

Although with current insight, there are conceivable situations that give reason for reconsideration of dosing regimens, writing this review raises many questions that need to be unravelled.

Data from several in vitro studies showed destabilization of corrected F508del CFTR and reduction of mature wild-type CFTR levels and function by too high ivacaftor concentrations [16-18]. Further research is needed to elucidate if this effect also occurs in vivo and to determine the ivacaftor concentrations giving rise to these negative effects. Organoid models may be helpful to determine the maximal effect concentration of ivacaftor (lower bound of therapeutic window) and to get insight in the concentration above which the negative effect on the corrector takes place (upper bound of therapeutic window).

In vitro studies have been performed to improve the insight in pharmacokinetic and –dynamic properties of CFTR modulators by investigating cellular concentrations of ivacaftor and measuring serum levels [21, 22]. More studies on plasma concentrations of CFTR modulators are needed to detect interindividual differences, interactions, and to be able to relate exposure to clinical efficacy and side effects. Besides studies on the relation between plasma and tissue concentrations, investigation of concentration effect relationship in organoids can be helpful to define a therapeutic window.

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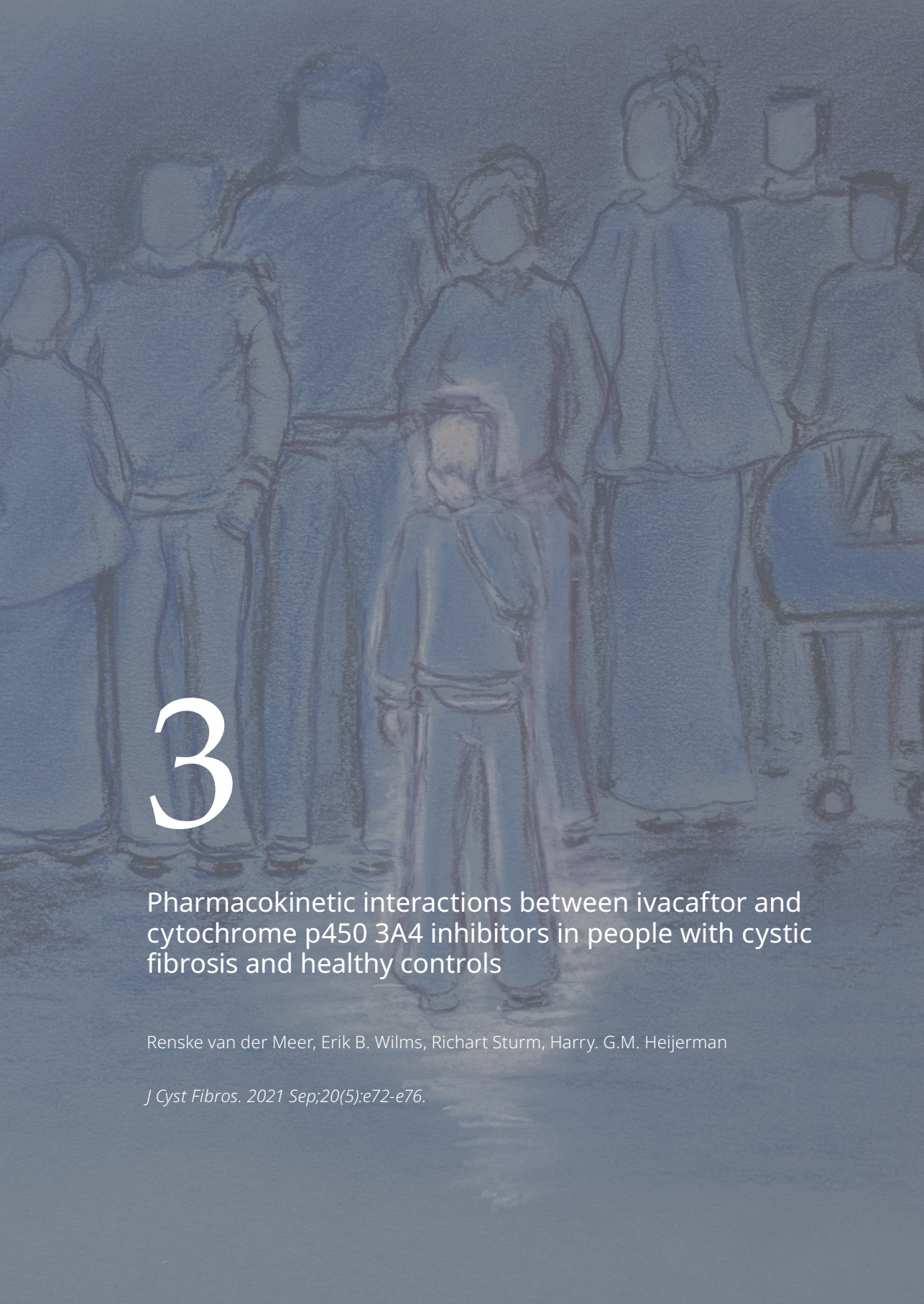

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Pharmacokinetic interactions between ivacaftor and cytochrome p450 3A4 inhibitors in people with cystic fibrosis and healthy controls

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Abstract

Background: Ivacaftor is currently the only CFTR potentiator approved and is increasingly used since the development of CFTR correctors. Ivacaftor is metabolized by CYP3A4 and therefore dose reduction is required when treating patients on ivacaftor with CYP3A4 inhibiting drugs. As this advice is based on studies in healthy volunteers and not in cystic fibrosis (CF) patients, we need to investigate this in both groups to be able to extrapolate these data to CF.

Methods: A cohort of CF patients and healthy subjects were exposed to a single dose of ivacaftor in combination with a strong (ritonavir), moderate (clarithromycin) and mild (azithromycin) CYP3A4 inhibitor. Ivacaftor concentrations were measured in all blood samples in order to calculate the pharmacokinetic parameters for ivacaftor.

Results: We found that exposure to ivacaftor was higher in healthy volunteers than in subjects with CF. However this difference was not statistically significant. No differences were observed in the interaction potential of CYP3A4 inhibitors between both study groups. The strong CYP3A4 inhibitor ritonavir, increased exposure to ivacaftor 7 times.

Conclusion: Our data support current recommendations for dose adjustment of ivacaftor in case of co-treatment with CYP3A4 inhibitors in people with CF. However, exposure to ivacaftor was higher in healthy subjects than in CF patients. Further study is needed to investigate the cause and implication of this difference.

Introduction

Cystic Fibrosis (CF) is the most common life-shortening hereditary disease in the Caucasian population and is caused by mutations in the gene that encodes for a protein called the cystic fibrosis transmembrane conductance regulator (CFTR) [1, 2]. This protein, an epithelial chloride channel, has important regulatory functions in various organs. Absence or dysfunction of this chloride channel causes symptoms in multi organ systems. Therefore, multiple drugs are needed to treat this complicated disease. Most therapies for CF treat the secondary consequence of the disease. In just the past few years, compounds have been identified that target mutation-specific defects of the CFTR gene [3]. Ivacaftor was the first CFTR modulator available for clinical use and is currently the only CFTR potentiator approved [4]. Ivacaftor facilitates increased chloride transport by potentiating the channel open probability (or gating) of CFTR protein located at the cell surface. Ivacaftor is also registered for clinical use in combination with CFTR correctors lumacaftor, tezacaftor and recently approved by the FDA and EMA as part of triple therapy which is a combination of ivacaftor, tezacaftor and elexacaftor [5-8]. Ivacaftor alone has demonstrated a clinically relevant effect in people with class III and class IV mutations (R117H) [9, 10].

The dosing advice for ivacaftor is 150 mg twice daily. Ivacaftor is metabolized by CYP3A4 in the active metabolite hydroxymethyl-ivacaftor (M1) and the inactive metabolite ivacaftor-carboxylate (M6). Co-administration of CYP3A4 inhibitors will affect the pharmacokinetics of ivacaftor. Therefore, dose reduction is required when co-administering CYP3A4 inhibitors with ivacaftor. Current advices for dose adjustment are based on two phase 1 studies in healthy male subjects, and not in CF patients, investigating the interaction between ivacaftor with ketoconazole (VX08-770-006 study) and fluconazole (VX 09-770-010 study). Regarding four important pharmacokinetic principles: absorption, distribution, metabolism and excretion (ADME) of a drug, CF patients differ from healthy subjects [11, 12, 13]. Therefore, it is important to study drug-drug interactions in patients with CF and healthy subjects to be able to extrapolate these data to CF patients.

As patients with CF are often treated with drugs that inhibit the activity of cytochrome P450 3A4, learning more about their interaction with ivacaftor is of great importance. In this study we will investigate the interaction of ivacaftor and three CYP3A4 inhibitors. Ritonavir, which is often used in drug-drug interaction studies as the golden standard for a strong CYP3A4 inhibitor. Clarithromycin, which is supposed to be a mild CYP3A4 inhibitor but considered a strong CYP3A4 inhibitor according to the current dose adjustment advice in combination with ivacaftor. And third, azithromycin because of

the major clinical relevance in CF patients. By investigating the interaction between ivacaftor and these CYP3A4 inhibitors in healthy controls and people with CF, we want to provide a well-founded dosing advice.

Methods

Study design

In a single-centre, open label, exploratory, intervention-study we exposed a cohort of CF patients and healthy subjects to a single dose of ivacaftor in combination with three CYP3A4 inhibitors. The study consisted of five phases. Between each intervention phase (phase 2-5) a wash out of at least 1 week was scheduled, covering at least 5 times $T_{1/2}$ of the administered inhibitor. Azithromycin being the final inhibiting agent due to its prolonged $T_{1/2}$. In phase 1, the informed consent procedure was performed, blood was collected (renal and liver function). All regular medication was continued except from azithromycin which was temporarily stopped at least 4 weeks prior to start of the interaction study. In phase 2, subjects received a single dose of 150 mg ivacaftor at site. Blood was collected at fixed time points after administration of ivacaftor (0h, 30 min, 1 h, 2h, 4h, 6h, 8h, 24h, 48h). In phase 3 subjects received 7 doses of ritonavir (during 3,5 days twice daily one 100mg tablet). The final dose of ritonavir and the single dose of 150mg ivacaftor were administered in the hospital and blood was collected just before and at fixed time points after the administration of ivacaftor (0h, 30 min, 1h, 2h, 4h, 6h, 8h, 24h, 48h and 72h). In phase 4 subjects received 5 doses of clarithromycin (during 2,5 days twice-daily one 500mg tablet). The final dose of clarithromycin and the single dose of 150mg ivacaftor were administered in the hospital and blood was collected just before and at fixed time points after the administration of ivacaftor (0h, 30 min, 1h, 2h, 4h, 6h, 8h, 24h, 48h and 72h). In phase 5 subjects received 3 doses of azithromycin (during 2,5 days subjects received a dose of 500mg). The final dose of azithromycin and the single dose of 150 mg ivacaftor were administered in the hospital. Blood was collected just before and at fixed time points after the administration of ivacaftor (0h, 30 min, 1 h, 2h, 4h, 6h, 8h, 24h, 48h). A schematic overview of the study design is shown in figure 1.

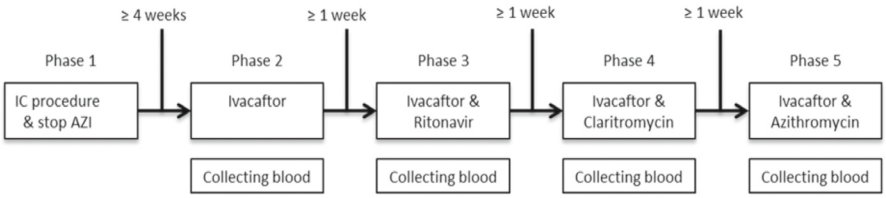

Figure 1. *Study overview.*

IC: informed consent, AZI: azithromycin

Before each phase, a pregnancy test was performed in female participants. After each phase and four weeks after the last study visit, all participants were asked for any adverse or serious adverse events or change in medication. Ivacaftor was taken together with a standardized fat containing snack.

Sample preparation

Blood was collected in a serum tube. After centrifugation serum samples were stored at -70 degrees. In all samples, serum concentrations of ivacaftor were measured, 100 µl serum was transferred into a 2 ml autosampler vial. 900 µl ice cold internal standard (ivacaftor C13-isotope in acetonitrile/methanol 85/15 v.v.) was added and mixed for 30 seconds. After 10 minutes of centrifugation, LC-MS/MS measurements were performed on the samples and analyzed in duplicate.

Blank bovine serum was spiked with known concentrations of ivacaftor, ivacaftor-M1 and ivacaftor-M6 to produce standard curves from 50 to 2500 µg/L (IVA), 20 to 1150 µg/L (IVA-M1) and 12.5 to 650 µg/L (IVA-M6).

LC-MS analysis

The LC-MS analyses were performed on an Agilent 1290 ultra performance liquid chromatography system (UPLC) with a directly coupled Agilent 6460 triple quadruple mass spectrometer (MS). UPLC equipment consisted of a G1316C thermostatted column compartment, a G4220B binary pump and a G4226A autosampler. Separation was obtained on a Zorbax Eclipse Plus C18 column (2.1x50 mm, 1.8 µm). Mobile phase A consisted of 0,1% Heptafluorobutyric acid (HFBA) in water and mobile phase B consisted of 0,1% formic acid in acetonitrile. The column temperature was 50°C. Injection volume was 0,5 µl and the flowrate 0,6 ml/min. The total run time was 2.5 minutes.

MS settings were as follows: positive electron spray mode, capillary voltage 4000V, drying gas (N2) 9 l/min at 350°C and nebulizer gas (N2) pressure 20 psi. Ivacaftor and metabolites were detected using multiple reaction monitoring (MRM). The following ion transitions were monitored: m/z 393.2 → 172.0 for ivacaftor, m/z 399.2 → 178.0 for ivacaftor-isotope, m/z 409.2 → 172.0 for ivacaftor-M1, and m/z 423.2 → 172.0 for ivacaftor-M6.

A limit of quantitation of 25 ug/L was obtained for ivacaftor and 20 ug/L for IVA-M1 and IVA-M6. For ivacaftor the lack of fit for a quadratic curve within the range of 50 – 2500 ug/L was 0.01. For IVA-M1 (range 22.5 – 1150 ug/L) the LOF was 0.02 and for IVA-M6 (range 12.5 – 625 ug/L) 0.01. The ivacaftor intraday variation was 3.8 – 1.3 – 2.5% and the interday variation was 4.7 – 3.6 – 4.9% for 25 – 500 – 3750 ug/L respectively.

Subjects

Subjects included in this study were patients with CF of 18 years and older with a class I or II CFTR gene mutation. Exclusion criteria were: liver cirrhosis, portal hypertension, severe renal impairment, use of drugs that are metabolised by CYP3A4 or with known influence on CYP3A4, allergy to study medication, pregnancy, lactation, pregnancy wish or a pulmonary exacerbation within one month before the study. The control group consisted of healthy volunteers of 18 years and older, with no use of medication, no pregnancy or pregnancy wish and not being a blood relative of a patient with cystic fibrosis.

Pharmacokinetic and statistical analysis

The pharmacokinetic parameters for ivacaftor were determined by non-compartimental methods using PK Solver version 2.0 [14]. Parameters estimated were area under the curve ($AUC_{0\text{-inf obv}}$), maximum plasma concentration (C_{max}), time to C_{max} (T_{max}) and terminal half-life ($T_{1/2}$). AUC was calculated using the linear trapezoidal rule. In order to calculate the effect of co-administration of the inhibitor, a ratio $AUC_{t\text{-}\infty}$ ($RAUC_{t\text{-}\infty}$) was calculated by dividing the $AUC_{t\text{-}\infty}$ of ivacaftor with co-administration of inhibitor by the $AUC_{t\text{-}\infty}$ of ivacaftor with no inhibitor.

Because of the observational character of this study and the high burden of this study for subjects, we chose to perform an exploratory study in 6 healthy subjects and 6 subjects with CF.

Statistical analysis of data from pharmacokinetic parameters derived from PK Solver Software were performed by using SPSS version 24 FP2. Pharmacokinetic parameters of ivacaftor were compared between healthy volunteers and CF patients using the

Mann-Whitney U test. A P value of <0.05 was considered significant. The change of ivacaftor pharmacokinetic parameters after treatment with the different CYP3A4 inhibitors was calculated in both groups by using the Wilcoxin signed rank test. A p value below 0.05 was considered significant.

Ethical considerations

The study was approved by the ethical review board "Medisch Etische Toetsings Commissie Zuid-West Holland". The study is in agreement with the Declaration of Helsinki. Healthy volunteers and CF patients were recruited through posters in the outpatient waiting room of the pulmonary department from the Haga Teaching Hospital. Questions about the study were answered by the treating physician or a member of the study team. The voluntariness of participation was underlined. Subjects all signed informed consent.

Results

Study subjects

Six healthy volunteers and five CF patients completed the study. Subject characteristics are summarized in table 1. All CF patients were pancreatic insufficient and all used pancreatic enzymes and proton-pump inhibitors.

Table 1. *Subject characteristics.*

	Healthy volunteers (N=6)	CF patients (N=5)
Race (percentage Caucasian)	100	100
Gender (percentage male)	50	20
Age (years) median (min-max)	37 (24-58)	27 (22-48)
Body weight (kg) median (min-max)	68,5 (58-91)	52,9 (48,7 – 62,4) *
Body mass index median (min-max)	23,1 (20,8-25,03)	20,9 (19,3-21,9)
Body surface area (m^2) median (min-max)	1,87 (1,58-2,22)	1,52 (1,46-1,71)

**significant difference between both groups P= 0.04.*

Safety and tolerability

In both study groups, ivacaftor and the CYP3A4 inhibitors were tolerated well. In the healthy volunteers no serious adverse events were reported. Six mild adverse events were reported by three subjects, common cold (n=1), nosebleed (n=1), upper airway

infection (n=1), fatigue (n=1), diarrhoea (n=1) and stomach ache (=1), all of them were deemed to be unrelated to the study medication. In the subjects with CF two serious adverse events were reported in one patient, both due to pulmonary exacerbations which were deemed to be unrelated to the study medication. Five patients reported nine mild adverse events including, viral airway infection (n=2), headache (n=1), stomach ache (n=1), sleeplessness (n=1), nausea (n=1), vomiting (n=1), common cold (n=2). The subjects recovered from the pulmonary exacerbations after antibiotic treatment. Other adverse events resolved spontaneously.

Concentration time profiles of ivacaftor in healthy volunteers and CF patients

The concentration–time profiles of ivacaftor in healthy volunteers and CF patients are shown per subject in figure 2a and 2b.

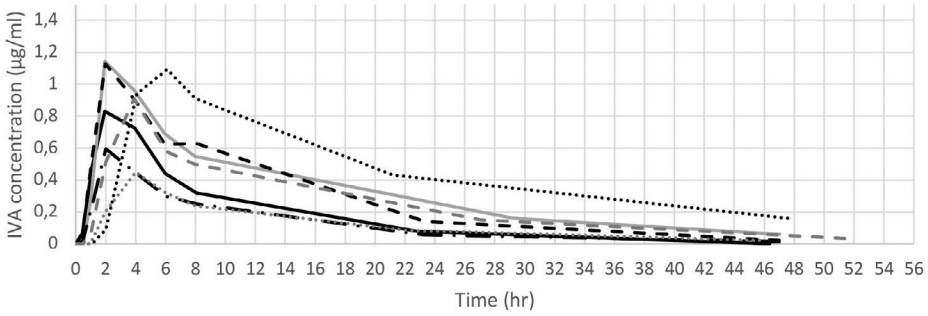

Figure 2a. *Concentration–time profiles of ivacaftor in healthy volunteers.*

Each line represents one individual

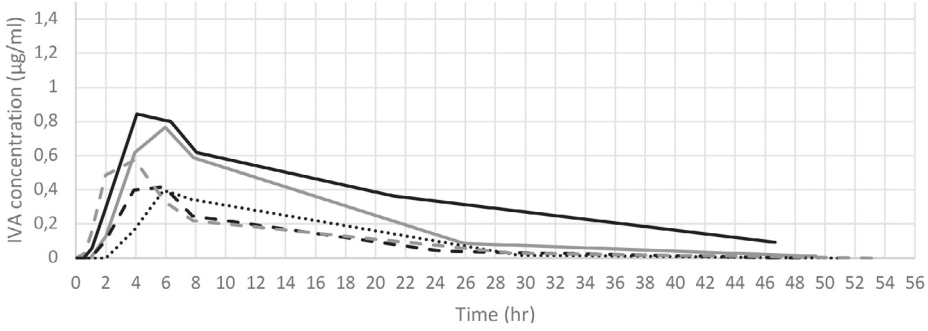

Figure 2b. *Concentration–time profiles of ivacaftor in CF patients.*

Each line represents one individual

The exposure to ivacaftor ($AUC0_{-inf\ obv}$) was approximately two times higher in healthy volunteers compared to CF patients, although the difference was not statistically significant due to wide individual variation. Pharmacokinetic parameters of ivacaftor in both groups are presented in table 2. Metabolites of ivacaftor M1 and M6 (not shown here) were also higher in healthy volunteers than in CF patients, but not significantly different.

Table 2. *Pharmacokinetic parameters of ivacaftor in healthy volunteers and CF patients.*

	Healthy volunteers (N=6)	CF patients (N=5)	P value
$AUC_{0\text{-}inf\ obv}$ (µg.hr.ml_1) median (min-max)	10,7 (6,04-25,27)	5,7 (4,75-18,55)	0,20
$T_{1/2}$ (hr) median (min-max)	9,9 (6,9-15,26)	6,8 (5,21-13,45)	0,10
T_{max} (hr) median (min-max)	2,96 (1,95-6,12)	5,87 (3,95-5,95)	0,10
C_{max} (µg/ml) median (min-max)	0,87 (0,45-1,13)	0,58 (0,40-0,84)	0,10

P values for difference between healthy volunteers and CF patients were measured with Mann-Whitney U test. p< 0.05 was considered significant.

Influence of CYP3A4 inhibitors on ivacaftor pharmacokinetic parameters in healthy volunteers and CF patients

Pharmacokinetic parameters of ivacaftor alone and in combination with CYP3A4 inhibitors are summarized in table 3.

Table 3. *PK parameters of ivacaftor alone and in combination with different CYP3A4 inhibitors for both groups.*

	Healthy volunteers (N=6)	CF patients (N=5)
Ivacaftor		
$AUC_{0\text{-inf obv}}$(ug.hr.ml_1) ivacaftor median (min-max)	10,7 (6,04-25,27)	5,7 (4,75-18,55)
$T_{1/2}$(hr) ivacaftor median (min-max)	9,90 (6,94-15,26)	6,8 (5,21-13,45)
T_{max} (hr) ivacaftor median (min-max)	2,96 (1,95-6,12)	5,87 (3,95-5,95)
C_{max} (µg/ml) ivacaftor median (min-max)	0,87 (0,45-1,13)	0,58 (0,40-0,84)
Ritonavir-ivacaftor		
R_{AUC} median (min-max)	8,86 (6,06-16,22)	6,63 (4,61-13,62)
$AUC_{0\text{-inf obv}}$(ug.hr.ml_1) median (min-max)	106,79 (54,74-153,25)	61,90 (37,22-123,05)
$T_{1/2}$(hr) median (min-max)	26,11 (22,01-49,18)	14,8 {(7,97-37,81)
T_{max} (hr) median (min-max)	4,38 (3,97-7,95)	5,9 (4,02-8,01)
C_{max} (µg/ml) median (min-max)	2,03 (1,10-2,89)	1,4 (1,38-2,02)
Clarithromycin-ivacaftor		
R_{AUC} clarithromycin median (min-max)	2,93 (1,13-6,70)	3,17 (1,87-3,85)
$AUC_{0\text{-inf obv}}$(ug.hr.ml_1) clarithromycin median (min-max)	36,52 (15,00-67,62)	20,27 (15,07-58,82)
$T_{1/2}$(hr) clarithromycin median (min-max)	11,16 (9,38-26,25)	8,79 (7,71-12,24)
T_{max} (hr) clarithromycin median (min-max)	3,99 (2,05-6,00)	4,10 (3,95-8,05)
C_{max} (µg/ml) clarithromycin median (min-max)	1,40 (0,91-2,09)	1,19 (0,92-1,86)
Azithromycin-ivacaftor		
R_{AUC} azithromycin median (min-max)	1,32 (0,55-3,33)	1,18 (0,67-1,40)
$AUC_{0\text{-inf obv}}$ (ug.hr.ml_1) azithromycin median (min-max)	13,02 (4.36-44,38)	6,66 (3,82-15,50)
$T_{1/2}$ (hr) azithromycin median (min-max)	10,18 (7.48-17,84)	6,38 (5,51-13,31)
T_{max} (hr) azithromycine median (min-max)	3,97 (1,95-6,00)	4,06 (3,97-6,03)
C_{max} (µg/ml) azithromycin median (min-max)	1,12 (0,52-1,57)	0,47 (0,40-1,01)

Ritonavir

The Ratio AUC (R_{AUC}) showed higher exposure to ivacaftor in the presence of ritonavir. R_{AUC} was 8,86 in healthy volunteers and 6,63 in CF patients. Comparing the $AUC_{0\text{-inf obv}}$, when ivacaftor was administered with ritonavir to the $AUC_{0\text{-inf obv}}$ of ivacaftor alone, a significant difference was seen (p=0,028 in healthy volunteers and p=0,043 in CF patients). In healthy volunteers, $T_{1/2}$, T_{max} and C_{max} of ivacaftor were significantly higher in the combination with ritonavir compared to ivacaftor alone (p=0,028; p=0,027 and p=0,028 respectively). In CF patients $T_{1/2}$ and C_{max} were significantly increased with p values of 0,043.

Clarithromycin

The exposure to ivacaftor was increased in the presence of claritromycin (R_{AUC} of 2,93 in healthy volunteers and 3,17 in CF patients). The $AUC_{0\text{-inf obv}}$ for ivacaftor in combination with clarithromycin was significantly higher than ivacaftor only (p value in healthy volunteers 0,028 and in CF patients 0,043). $T_{1/2}$ and C_{max}, but not T_{max}, were significantly higher when ivacaftor was administered with clarithromycin in healthy volunteers (respectively: P values of 0,046 and 0,028) and in CF patients only T_{max} differed significantly (p=0,043).

Azithromycin

Administering ivacaftor in subjects using azithromycin only, slightly increased the exposure to ivacaftor (R_{AUC} of 1,32 in healthy subjects and 1,18 in CF patients) without statistical significance.

The change in exposure to ivacaftor due to co-administration of ritonavir, clarithromycin and azithromycin was not significantly different between healthy subjects and people with CF.

Discussion

This pharmacokinetic trial showed remarkable differences in the pharmacokinetic parameters of ivacaftor between subjects with CF and healthy volunteers. No differences between CF patients and healthy subjects were observed in the interacting potential of CYP3A4 inhibitors. Although not statistically significant, the exposure ($AUC_{0\text{-inf obv}}$) to ivacaftor was almost two times lower in CF patients, with a higher T_{max} and a lower C_{max} and $T_{1/2}$ suggesting a more gradual uptake of ivacaftor in CF patients and a reduced bio-availability. Also, CF patients tend to have more lean body mass per kilogram bodyweight. This means that CF-patients have a larger volume of distribution

than non-CF-patients. The strong CYP3A4 inhibitor ritonavir, increased the exposure to ivacaftor approximately 7-8 times. The moderate inhibitor, clarithromycin, increased exposure 3 times and the mild inhibitor, azithromycin did not significantly change the exposure to ivacaftor.

Our pharmacokinetic data of ivacaftor are comparable with the summary of product characteristics (SmPC) (mean AUC for ivacaftor in healthy volunteers 10,6 ug.h.ml_1 and C_{max} 0,77 ug/ml) [15]. In the SmPC an 8,5 times increased exposure to ivacaftor is described after co-administration of a strong CYP3A inhibitor in healthy subjects. Our results show the same amount of increase, also in CF patients. In the SmPC, clarithromycin is considered a strong inhibitor with the same dosing advice as for ritonavir or ketoconazole. However, the results of our study show a much smaller effect on ivacaftor exposure than ritonavir (3 versus 7-8 times) compatible with moderate instead of strong CYP3A4 inhibition. In a study from Liddy et al. [16] the effect of ritonavir on the exposure to ivacaftor was studied. They found a 20 times higher exposure to ivacaftor while co-administered with ritonavir, which is much more than in our study and the SmPC.

Despite an almost two fold higher exposure to ivacaftor in healthy volunteers, the difference did not reach statistically significance. This may be due to the relatively small number of subjects, non- parametric testing and high level of variation in the AUC. However, by using a non-parametric test we possibly have underestimated the power of the results which are obtained by repeated measurements within one subject. Baseline characteristics showed a statistically significant higher weight in the healthy subjects compared to the CF patients. Because this difference in weight might confound our results we analysed if body weight and body surface area were correlated with AUC of ivacaftor in both study groups, which was not the case (R 0,5 and 0,6 respectively). All subjects with CF were pancreatic insufficient, they all used proton pump inhibitors. We did not standardize the dose of pancreatic enzymes. The variance in dosing might have influenced the absorption of ivacaftor. We did not investigate the reproducibility of the measurements within one patient. This may be important regarding the high variation of pharmacokinetic parameters within one study group. We plan to investigate the reproducibility of pharmacokinetic parameters in a separate study.

Regarding our results, the dosing advice as mentioned in the SmPC for ivacaftor during the concomitant use of CYP3A4 inhibitors in patients with CF can be maintained. Concomitant use of a strong CYP3A4 inhibitor a dose adjustment to 150 mg twice a week and with a mild CYP3A4 inhibitor 150 mg once daily. However, the advice for the

combination with clarithromycin should change to 150 mg once daily. The variance in exposure to ivacaftor within both study groups was remarkably high. Therefore, insight in the relation between blood concentrations and clinical effect of ivacaftor would be interesting but is currently unclear.

In conclusion, recommendations for dose adjustment when co-administrating CYP3A4 inhibitors with ivacaftor, are based on studies in healthy subjects. With our study we showed that this advice can be extrapolated to CF patients. We found a trend to a higher exposure to ivacaftor in healthy volunteers, but this will not change the dosing advice.

Authorship contribution statement

Renske van der Meer: Methodology, Formal analysis, Investigation, Resources, Writing – original draft, Writing – review & editing, Visualization. **Erik B Wilms:** Methodology, Validation, Formal analysis, Writing – review & editing, Visualization. **Richart Sturm:** Validation, Formal analysis, Investigation, Resources. **Harry G.M. Heijerman:** Conceptualization, Methodology, Writing – review & editing, Supervision.

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4

The influence of exocrine pancreatic function on the exposure and pharmacokinetics of ivacaftor in people with cystic fibrosis

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Abstract

Background: Cystic fibrosis transmembrane conductance regulator (CFTR) modulator therapies target the underlying cause of cystic fibrosis (CF), and show robust treatment effects at group level. The individual effect however, is variable which might be (partially) related to differences in drug exposure. The profound influence of fat containing food compared to fasting on drug exposure gives need to investigate if the exocrine pancreatic function changes the degree and rate of absorption of ivacaftor and thereby may contribute to differences in drug exposure.

Methods: Pharmacokinetic parameters of ivacaftor were measured in 10 pancreatic sufficient (PS) and 10 pancreatic insufficient (PI) patients with CF on current treatment with tezacaftor/ivacaftor and compared between both groups. In PI patients pharmacokinetic parameters were investigated with and without the use pancreatic enzymes and compared in each individual.

Results: We demonstrated that the pharmacokinetic parameters of ivacaftor did not differ significantly between PS and PI people with CF (pwCF). Pancreatic enzymes did not significantly change the absorption or exposure to ivacaftor in PI pwCF using tezacaftor/ivacaftor.

Conclusion: The exocrine pancreatic function of pwCF does not significantly influence the absorption and exposure of ivacaftor. The use of pancreatic enzymes in PI pwCF does not change the absorption and exposure of ivacaftor. Therefore, the dosing advice as mentioned in the SmPC for ivacaftor can be maintained independent of the exocrine pancreatic function.

Introduction

Over the past decade, cystic fibrosis transmembrane conductance regulator (CFTR) protein modulators have been registered for cystic fibrosis (CF) depending on the genotype. These modulators improve CFTR function either through potentiation of the abnormal protein channel at the cell surface (ivacaftor), or through correction of protein transport to the cell surface (lumacaftor, tezacaftor, elexacaftor). Although these (combination of) modulators show robust treatment effects at group level the individual effect is variable [1-4]. In the supplementary figures, Wainwright et al. showed a change from baseline in $ppFEV_1$ ranging from -10% to >+10% after 24 weeks of treatment with lumacaftor/ivacaftor [3]. Heijerman et al., showed the individual response to elexacaftor/tezacaftor/ivacaftor treatment in the supplementary figures, with a change in $ppFEV_1$ ranging from -2.5% to > +20% [1].

The effect of CFTR modulators in an individual patient is a result of what the drug does to the body (pharmacodynamics) and what the body does to the drug (pharmacokinetics). Different features of CF disease may influence pharmacokinetic properties. Administered as an oral dose, CFTR modulators are absorbed directly from the gut. It is advised to take CFTR modulators in combination with fat containing food because the bioavailability increases two to four times compared to a fasting state (except for tezacaftor) [5-8]. Ivacaftor itself is a lipophilic, hydrophobic molecule with low water solubility (<0,05 µg/ml) [5]. In a recent study, we investigated the pharmacokinetics of ivacaftor in people with CF (pwCF) and healthy volunteers [9]. Our data showed an almost twofold higher exposure to ivacaftor in healthy people than in pwCF. PwCF showed a delayed T_{max} and a lower C_{max} and lower $T_{1/2}$ suggesting a slower and lesser degree of absorption. All pwCF in this study suffered from exocrine pancreatic insufficiency (PI). Despite treatment with pancreatic enzymes PI pwCF still suffer from fat malabsorption [10]. The profound influence of fat containing food compared to fasting and the reduced level of exposure in PI pwCF compared to healthy volunteers stresses the need for knowledge of the influence of pancreatic function and pancreatic enzymes on the absorption (and exposure) of ivacaftor. Tezacaftor/ivacaftor is registered for pwCF homozygous for the508del mutation [11] and for pwCF heterozygous for the F508del mutation and a CFTR residual function mutation [4]. PwCF and a CFTR residual function mutation are often pancreatic sufficient which allows us to investigate the pharmacokinetic parameters of the same drug in both pancreas sufficient and insufficient pwCF. Currently, the dosing advice for all CFTR modulators is the same for PI and pancreatic sufficient (PS) pwCF. We hypothesize that the exocrine pancreatic function changes the degree and rate of absorption of ivacaftor and thereby may contribute to differences in drug exposure. With this

study we aim to gain insight in the influence of exocrine pancreatic function on the pharmacokinetics of ivacaftor, including the effect of pancreatic enzymes on the resorption pharmacokinetics of ivacaftor in pwCF using tezacaftor/ivacaftor.

Methods

Study design and subjects

A single-center, open label, exploratory, intervention study was performed in 10 PS pwCF and 10 PI pwCF who were on Symkevi treatment (tezacaftor/ivacaftor (100 mg/150 mg) in the morning, ivacaftor (150 mg) in the evening). Patients were aged 18 years or older. In case of PI, patients who were treated with Creon 10.000 units (amylase 8000 FIP-E, lipase 10.000 FIP-E and protease 600 FIP-E), were eligible. Patients were excluded from study participation in case of the use of drugs metabolized by the CYP3A4 enzyme (inducers or inhibitors),

a pulmonary exacerbation with hospital admission within one month before study participation and pregnancy or breastfeeding.

Study procedures

After patients volunteered to participate and signed the informed consent form, a screening visit was scheduled. During this visit eligibility to participate was verified, vital signs and fat free mass index (FFMI) were measured, and in PS patients a stool elastase test was performed to validate their current pancreatic sufficiency, which was defined as a value of ≥200 µg elastase per gram feces. PS patients with an elastase level below 200 µg were excluded from the study. After successful screening, PS patients visited the hospital once and PI patients twice. Patients were asked to register the time of administration of tezacaftor/ivacaftor and ivacaftor during 2 days before their study visit. Patients visited the hospital before taking their morning dose tezacaftor/ivacaftor and Creon (if applicable) and were asked to fast at least 4 hours prior to their study visit. During each study visit, patients took their morning dose tezacaftor/ivacaftor (100 mg/150 mg) in the hospital with a standardized fat containing breakfast (containing 20 grams of fat). At visit day 1, PI patients took a dose of 2 tablets of Creon 10.000 U in addition to one tablet of tezacaftor/ivacaftor and the standardized fat containing breakfast. PI patients did not use their Creon during the second study visit. At each study visit blood was collected at 7 fixed time points; before (T=0 min) and after administration of tezacaftor/ivacaftor (T=30 min, 1 h, 2 h, 4 h, 6 h and 8 h). PI patients received their usual dose of Creon with their lunch. To avoid confounding effects on the absorption of ivacaftor, all patients received their lunch after T 6h, which is the

Tmax of ivacaftor. Except for a fat free snack between breakfast and lunch, patients were not allowed to eat or drink anything other than water of coffee/tea during the study visit. An overview of the study interventions is shown in table 1.

Sample preparation

Blood was collected in a serum tube. After centrifugation serum samples were stored at -70 degrees Celsius. Serum concentrations of ivacaftor were measured in a 100 µl serum aliquot after addition of 900 µl ice cold internal standard (ivacaftor C13-isotope in acetonitrile/methanol 85/15 v.v.) and mixing for 30 seconds. After 10 minutes of centrifugation, LC-MS/MS measurements were performed on the samples and analyzed in duplicate. Blank bovine serum was spiked with known concentrations of ivacaftor to produce standard curves from 50 to 2500 µg/L.

LC-MS analysis

The LC-MS analyses were performed on an Agilent 1290 ultra performance liquid chromatography system (UPLC) with a directly coupled Agilent 6460 triple quadruple mass spectrometer (MS). UPLC equipment consisted of a G1316C thermostatted column compartment, a G4220B binary pump and a G4226A autosampler. Separation was obtained on a Zorbax Eclipse Plus C18 column (2.1×50 mm, 1.8 µm). Mobile phase A consisted of 0,1% Heptafluorobutyric acid (HFBA) in water and mobile phase B consisted of 0.1% formic acid (HFBA) in acetonitrile. The column temperature was 50°C. Injection volume was 0.5 µl and the flowrate 0.6 ml/min. The total run time was 2.5 minutes. MS settings were: positive electron spray mode, capillary voltage 4000V, drying gas (N2) 9 l/min at 350°C and nebulizer gas (N2) pressure 20 psi. Ivacaftor was detected using multiple reaction monitoring (MRM) on ion transitions: m/z 393.2 → 172.0. A limit of quantitation of 25 ug/L was obtained for ivacaftor. The lack of fit for a quadratic curve within the range of 50 – 2500 ug/L was 0.01. The ivacaftor intraday variation was 3.8 – 1.3 – 2.5% and the interday variation was 4.7 – 3.6 – 4.9% for 25 – 500 – 3750 ug/L respectively.

Pharmacokinetic and statistical analysis

The pharmacokinetic parameters for ivacaftor were determined by non-compartimental methods using PK Solver version 2.0 [12] and by assuming T12h=T0h in steady state. Parameters estimated were the area under the curve ($AUC_{0\text{-}12h}$), maximum plasma concentration (C_{max}), time to C_{max} (T_{max}) and terminal half-life ($T_{1/2}$). AUC was calculated using the linear trapezoidal rule. The primary intention of this study was to investigate differences between PS and PI patients in exposure and the pharmacokinetic parameters of ivacaftor. For the sample size calculation we used a power of 80%, an expected and relevant difference in AUC of 50% and a standard

deviation of 4. This is based on a previous study [9] in healthy volunteers (AUC was 10) and pancreatic insufficient CF patients (AUC was 5). The number of patients needed would be approximately 8 in both groups. Due to the possibility of preliminary withdrawal from the study or missing data, we included 10 patients in each group.

Ethical considerations

The study was approved by the ethical review board "Medisch Etische Toetsings Commissie Zuid-West Holland". The study is in agreement with the Declaration of Helsinki. Questions about the study were answered by the treating physician or a member of the study team. The voluntariness of participation was underlined. Patients all signed informed consent.

Table 1. *Overview of study visits and investigations.*

Interventions	Screening	Visit 1	Visit 2 (PI only)
Informed consent	x		
Check Eligibility criteria	x		
Medical history	x		
Stool elastase test (PS only)*	x		
Tezacaftor/ivacaftor administration		x	X
Pancreatic enzymes administration (PI only)		x	
Blood sampling		x	X
Pregnancy test (females)		x	X
Co-medication and adverse events registration		x	X
Vital signs, weight, length and BMI		x	X
Fat free mass index	x (or)	x (or)	x (or)

** performed after informed consent was signed.*

Results

Study subjects

10 PS pwCF and 10 PI pwCF completed the study. All patients were compliant in their modulator administration during the 2 days before their study visit. Age and body weight were significantly higher in PS patients than in PI patients. However, FFMI was not significantly different. Patient characteristics are summarized in table 2.

Table 2. *Patient characteristics.*

	PS (N=10)	PI (N=10)
Race (percentage Caucasian)	100	100
Gender (percentage male)	50	70
Age (years) median (min-max)	44,5 (26-64)	31 (24-61) *
Body weight (kg) median (min-max)	73 (59,3-93)	59,8 (49,5 – 82) #
FFMI kg/m^2 median (min-max)	16,2 (12,9-17,8)	17,3 (13,9-19,5) ^

** P= 0,02, #P=0,02, ^P=0,13.*

Safety and tolerability

The duration of the study per patient was short because all PI patients planned their study visits on two consecutive days. No adverse events or serious adverse events were reported.

Concentration time profiles of ivacaftor

The concentration-time profiles of ivacaftor in PS patients and PI patients with and without concomitant use of pancreatic enzymes are shown per group and per patient in figure 1a, 1b and 1c. Ivacaftor concentrations in PS patients (fig 1a) showed a large between patient variability. However, for 80% of the patients ivacaftor concentrations were between 0,5 and 2,5 µg/ml. Ivacaftor concentrations measured in PI patients with and without the use of pancreatic enzymes were within the same range of 0,5 to 2,5 µg/ml (fig 1b, 1c). Pharmacokinetic parameters of ivacaftor for the three groups are presented in table 3. The absorption and the exposure to ivacaftor (AUC0-12) was not significantly different in PS pwCF compared to PI pwCF with and without pancreatic enzyme suppletion.

The influence of pancreatic enzymes on ivacaftor absorption and exposure in PI pwCF

Pharmacokinetic parameters of ivacaftor in PI pwCF with and without pancreatic enzymes are shown in table 3. The use of pancreatic enzymes did not change the absorption nor the exposure to ivacaftor in the pancreatic insufficient pwCF. The Ratio C_{max} (Rc_{max}) is the C_{max} with pancreatic enzymes divided by the C_{max} without pancreatic enzymes. The mean Rc_{max} (min-max) was 1,09 (0,60 – 1,50). The Ratio AUC (R_{AUC}) is the AUC with pancreatic enzymes divided by the AUC without pancreatic enzymes. The mean R_{AUC} (min-max) was 1,04 (0,68 - 1,35). The concentration-time profiles per

pancreas insufficient individual with and without enzyme suppletion are shown in supplementary figure 1.

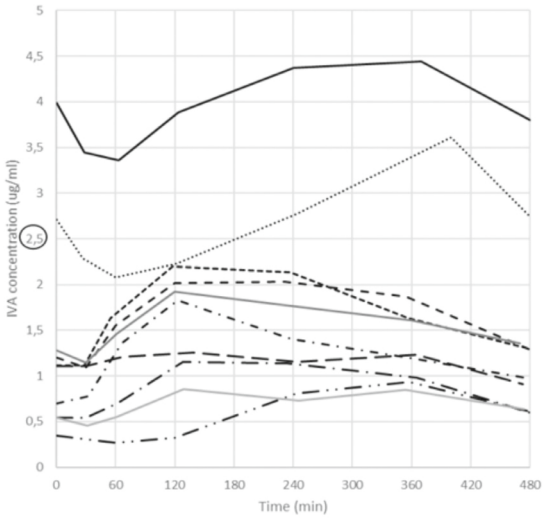


Figure 1a. *Concentration-time profiles of ivacaftor in pancreatic sufficient patients.*

Each line represents one individual

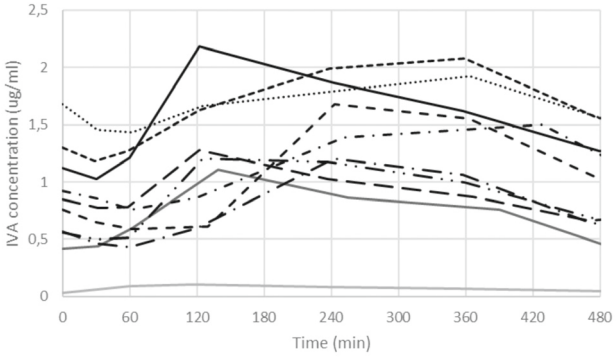


Figure 1b. *Concentration-time profiles of ivacaftor in pancreatic insufficient patients on visit day 1 (with pancreatic enzymes).*

Each line represents one individual

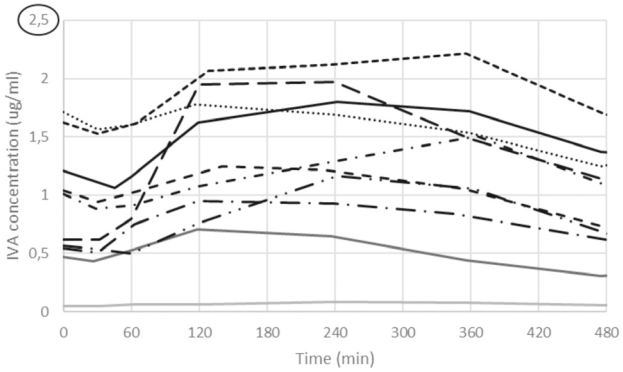


Figure 1c. *Concentration-time profiles of ivacaftor in pancreatic insufficient patients on visit day 2 (without pancreatic enzymes).*

Each line represents one individual

Table 3. *pharmacokinetic parameters of ivacaftor in PS pwCF, PI pwCF with pancreatic enzymes (visit 1) and PI pwCF without pancreatic enzymes (visit 2).*

	PS (N=10)	PI + enzymes (N=10)	PI – enzymes (N=10)	P values
$AUC_{0\text{-}12}$ (µg.hr.ml_1) median (min-max)	15,42 (6,93-48,05)	11,73 (0,80-20,18)	12,99 (0,78-22,40)	*0,41, #0,36,^0,51
$T_{1/2}$ (hr) median (min-max)	10,02 (1,51-51,49)	7,82 (6,24-40,18)	12,33 (4,77-68,89)	*0,36, #0,65, ^0,06
T_{max} (hr) median (min-max)	2,24 (1,97-6,67)	3,18 (1,95-7,13)	4,00 (1,95-6,10)	*0,85, #0,97, ^0,92
C_{max} (µg/ml) median (min-max)	1,88 (0,86- 4,44)	1,39 (0,10-2,18)	1,38 (0,08-2,21)	*0,43, #0,23, ^0,20

** PS versus PI + enzymes (Mann Withney test), # PS versus PI – enzymes (Mann Withney test), ^ PI+ enzymes versus PI- enzymes (Wilcoxon signed rank test). P<0,05 was considered significant.*

Discussion

To our knowledge, this was the first study to investigate the influence of the pancreatic function on the absorption and exposure of ivacaftor. We demonstrated that the pharmacokinetic parameters of ivacaftor did not differ significantly between PS and PI pwCF. Pancreatic enzymes did not significantly change the absorption or exposure to ivacaftor in PI pwCF.

Both PS and PI patients were on tezacaftor/ivacaftor treatment. Data from the SmPC [8] of tezacaftor/ivacaftor show steady state pharmacokinetic parameters in a fed state in patients with CF aged 12 years and older. The pancreatic function of these patients has not been reported. The following data for ivacaftor are mentioned in the SmPC [8] of tezacaftor/ivacaftor: AUC (mean(SD)) of 10,9 (3,9) µg.hr.ml_1, $T_{1/2}$ (mean) of 9 hr, T_{max} (median(range)) of 6 (3-10)hr and C_{max} (mean (SD)) of 1,3 (0,4) µg/ml. Although pharmacokinetic data in our study are in the same range, the abbreviated blood sampling scheme might have resulted in half life time values with a suboptimal accuracy. In a previous study [9], we found a lower exposure to ivacaftor in PI pwCF (median AUC of 5,7 µg.hr.ml_1) compared to healthy volunteers (median AUC of 10,7 µg.hr.ml_1). These data were measured after a single dose of ivacaftor, which explains the higher AUC in the current study performed in steady state. As mentioned in the SmPC [8] of tezacaftor/ivacaftor, the AUC of tezacaftor did not change when given with fat-containing food relative to fasted conditions. Therefore we supposed tezacaftor concentrations not to be affected by exocrine pancreatic function. As expected the absorption and exposure to tezacaftor was the same in PS and PI pwCF.

The power calculation of our study was based on data obtained in a previous study [9] with a wide variation in exposure between patients. Although we have achieved the target number of study participants, no significant differences in ivacaftor pharmacokinetic parameters were measured. The variation in AUC between the patients was high. Two PS patients had a very high exposure to ivacaftor (see fig. 1a), which we could not explain by patient characteristics. In one PI patient an extremely low ivacaftor exposure was measured on both study visits (fig 1b and 1c). This has probably been caused by the treatment with courses of very high doses of methylprednisolone 2 weeks before study participation. Pharmacokinetic parameters did not reach significant difference after exclusion of the outlier in the PI group. Possibly the differences in BMI may have influenced our results. However, the FFMI which is a more reliable parameter to investigate pharmacokinetics, was the same in both groups. Other potential confounding factors as albumin level, liver cirrhosis, use of co-medication e.g. proton pump inhibitors, were the same for both groups.

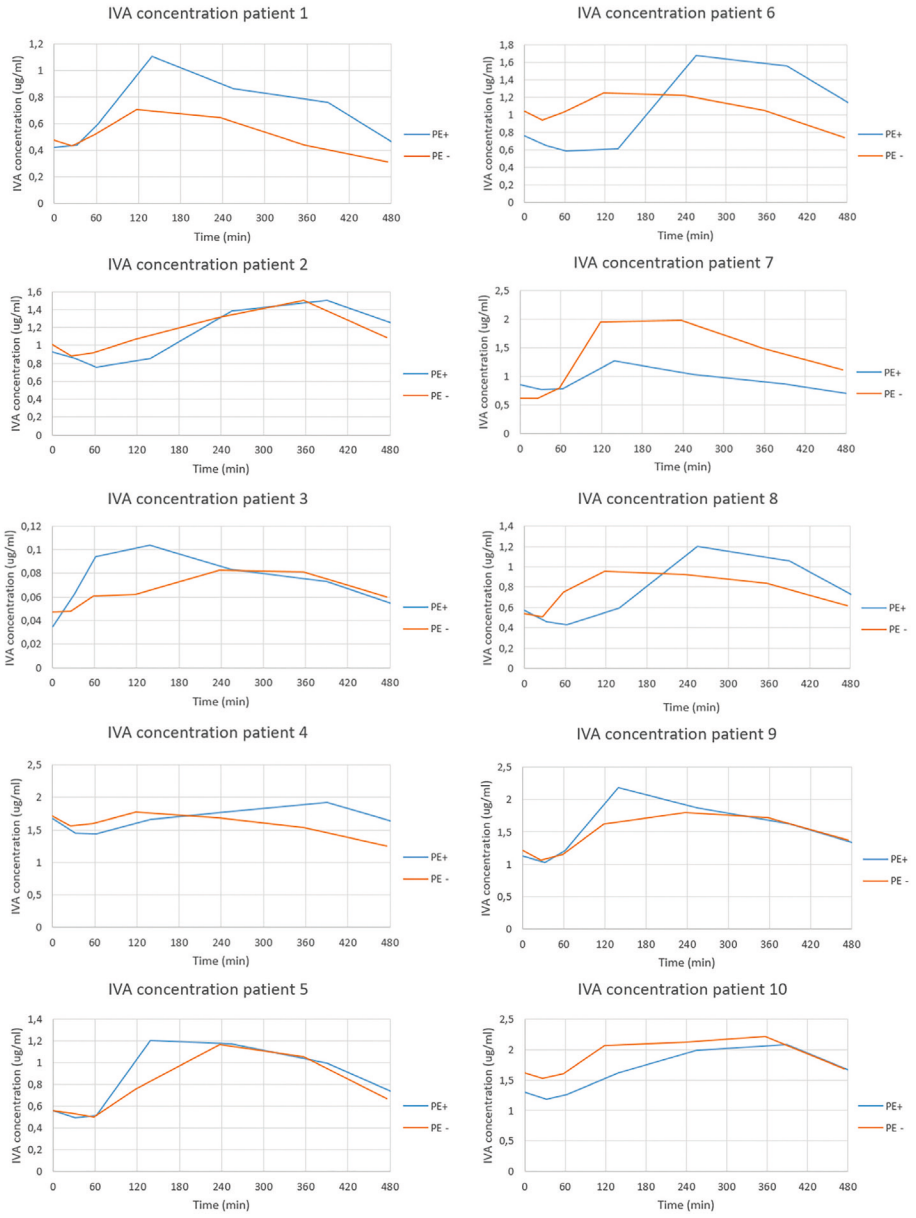
With our study we are able to investigate the reproducibility of trough levels within one patient in the PI patients on two consecutive days. In all patients these levels were reproducible making this unlikely to cause the high variation in pharmacokinetic parameters of ivacaftor in pwCF.

In the general population, the knowledge about drug absorption in people with exocrine pancreatic insufficiency is limited. Exocrine pancreatic insufficiency causes maldigestion which may also be associated with other changes in gastrointestinal physiology such as: changes in gastro intestinal intraluminal pH, motility disorders, bacterial overgrowth and pancreatic secretion [13].

However, our data show that PI pwCF do not differ significantly in the rate of absorption nor exposure of ivacaftor as compared to PS pwCF. All PI pwCF used proton pump inhibitors to increase their gastrointestinal intraluminal pH, which may have improved their absorption. Data on extrapulmonary effects of CFTR modulators is increasing. Recent studies showed that the use of CFTR modulators itself may improve fat absorption in PI pwCF [14-16]. Some reports suggest that pancreatic duct function and thereby pancreatic enzyme secretion can be restored by ivacaftor [17-19]. Most studies were performed in infants and young children with gating mutations. In an older population (ages 5–61 years), Stallings et al. found no change in faecal elastase between baseline and 3 months of ivacaftor therapy, but the coefficient of fat absorption increased significantly in PI pwCF [20]. However, the reversibility of fat malabsorption in older PI pwCF is uncertain.

The data obtained by our study gives no reason for a different dosing advice for ivacaftor in PI pwCF and PS pwCF. PI pwCF might not need to administer their pancreatic enzymes with their ivacaftor necessarily. As mentioned in the SmPC for ivacaftor the AUC is increased by 2-4 times when administering ivacaftor with fat containing food [8]. This advice is based on study data that are not publicly available. Because CFTR modulators are hydrophobic and lipophilic compounds, intake together with fat is expected to increase dissolution of ivacaftor and thereby enable the absorption. Therefore, the advice to administer these drugs with fat containing food is maintained regardless of the exocrine pancreatic function.

In conclusion, the exocrine pancreatic function of pwCF does not significantly influence the absorption and exposure of ivacaftor. The use of pancreatic enzymes in PI pwCF does not change the absorption and exposure of ivacaftor. Therefore, the dosing advice as mentioned in the SmPC for ivacaftor can be maintained independent of the exocrine pancreatic function.



Supplementary figure 1. *Concentration-time profiles with and without pancreas enzyme suppletion presented for each pancreas insufficient individual.*

PE+ with pancreas enzyme suppletion, PE- without pancreas enzyme suppletion.

Authorship contribution statement

Renske van der Meer: Methodology, Formal analysis, Investiga- tion, Resources, Writing –original draft, Writing –review & edit- ing, Visualization. **Erik B Wilms:** Methodology, Validation, Formal analysis, Writing –review & editing, Visualization. **Margot N Eggermont:** Investigation, Resources, Data curation, Writing –review & editing. **Helena M Paalvast:** Investigation, Resources, Data cura- tion. **Richard C J M van Rossen:** Validation, Formal analysis, Re- sources. **Harry G M Heijerman:** Concep tualization, Methodology, Writing –review & editing, Supervision.

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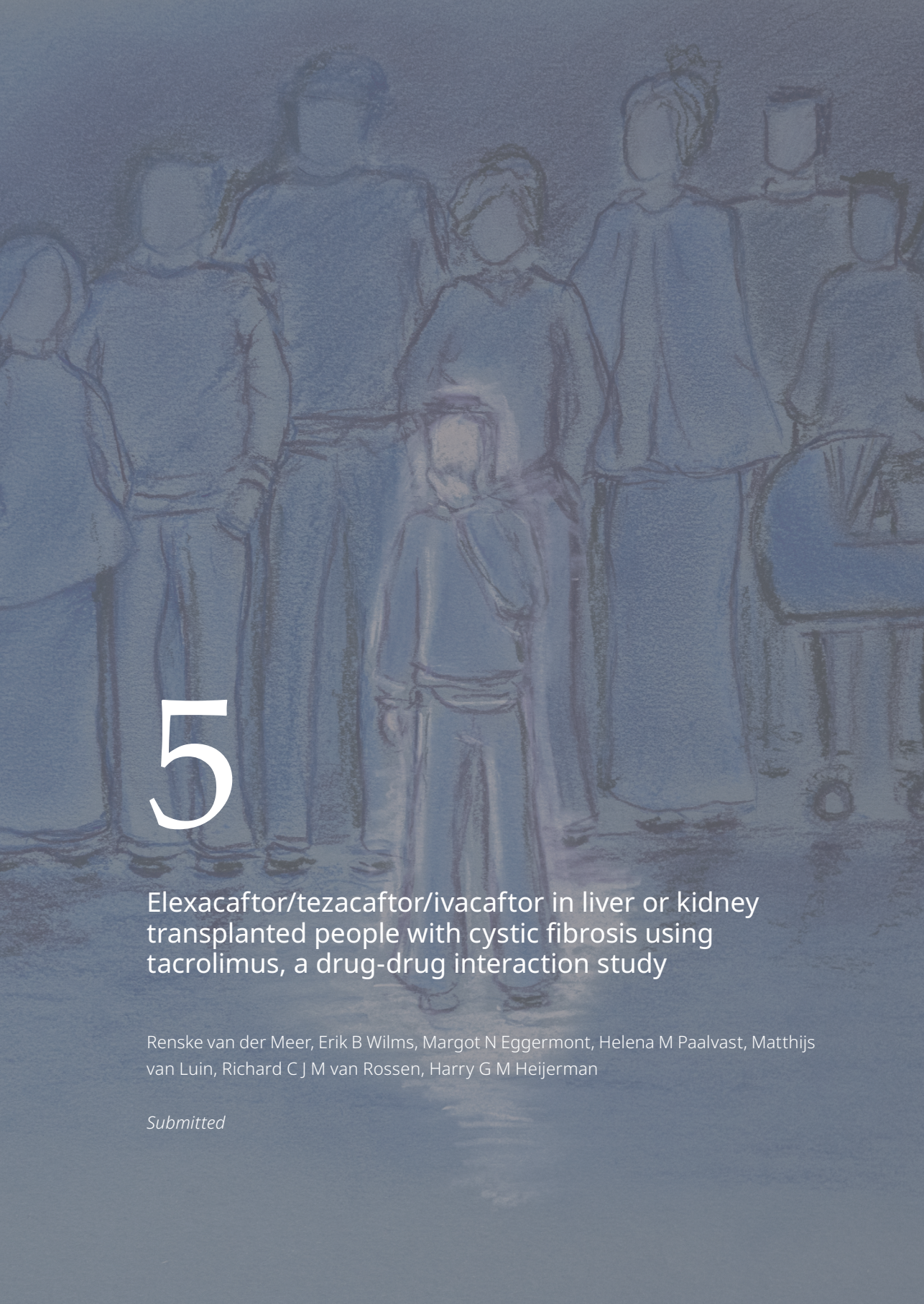

5

Elexacaftor/tezacaftor/ivacaftor in liver or kidney transplanted people with cystic fibrosis using tacrolimus, a drug-drug interaction study

Renske van der Meer, Erik B Wilms, Margot N Eggermont, Helena M Paalvast, Matthijs van Luin, Richard C J M van Rossen, Harry G M Heijerman

Submitted

Abstract

Background: The use of elexacaftor/tezacaftor/ivacaftor (ETI) in people with cystic fibrosis (pwCF) after solid organ transplantation is controversial because of potential drug-drug interactions (DDI) with tacrolimus. We aimed to improve insight into the safety and clinical benefits of co-administration of ETI and tacrolimus in liver or kidney transplanted adult pwCF.

Methods: In 5 pwCF, tacrolimus concentrations were monitored during 2 weeks before and 4 weeks after starting ETI treatment. Trough levels, area under the curve (AUC) and clinical effect of ETI were investigated. During the study (6 weeks in total) adverse events were monitored.

Results: The DDI between tacrolimus and ETI resulted in an increased exposure of tacrolimus in all subjects, the dose adjusted $AUC_{0\text{-}24h}$ was 1,79 (median) times higher at the end of the study. Five dose adjustments were performed in 4 subjects in order to attain tacrolimus target range. No adverse events were reported and all subjects showed clinical improvement during ETI treatment.

Conclusion: The clinical value of ETI treatment in kidney and liver transplanted pwCF is clear. The use of ETI may increase tacrolimus levels moderately. Therefore, we recommend close monitoring of tacrolimus trough levels in patients who start ETI.

Introduction

The combination of cystic fibrosis transmembrane conductance regulator (CFTR) modulators elexacaftor, tezacaftor and ivacaftor (ETI) has shown a life changing clinical effect in people with cystic fibrosis (pwCF) with at least one F508del mutation [1-3]. The use of ETI in pwCF after solid organ transplant is controversial because of potential drug-drug interactions (DDI) with tacrolimus, a first-line immunosuppressive agent and a substrate of CYP3A4 [4]. The inhibition of CYP3A4 and the P-gp inhibition by ivacaftor has a potential risk to increase the systemic exposure to tacrolimus [5].

Few studies show retrospective data of ETI in pwCF after organ transplantation [6-9)] A recent study used Physiologically Based Pharmacokinetic Modeling (PBPK) to estimate the DDI between ETI and tacrolimus [10]. Prospective clinical DDI studies are needed to determine the risks and benefits of ETI in pwCF after organ transplantation. Since the justification for using ETI in kidney and liver transplanted pwCF is clear, we conducted a prospective study, aiming to quantify the DDI and improve insight into the safety and clinical benefits of co-administration of ETI and tacrolimus.

Methods

A single-center, open label, clinical DDI study was performed in 5 pwCF with a history of kidney or liver transplantation ≥ 1 year ago, currently using tacrolimus and a CFTR mutation combination registered for the use of ETI.

The study started with a tacrolimus monitoring period of 2 weeks (day -14 till day 1). Subjects used their regular daily dosage of tacrolimus. ETI was started on day 1. On day -14, 1, 4, 11, 18 and 28 blood was collected before, 1 and 3 h after tacrolimus administration for limited AUC tacrolimus sampling and to determine serum concentrations of elexacaftor, tezacaftor and ivacaftor. Throughout the study, subjects were asked to take dry blood spot samples (DBS) three times a week for tacrolimus analysis before taking their tacrolimus.

Subjects were instructed to take their tacrolimus in a fasted state and 1h prior to ETI since the uptake of tacrolimus is decreased by food. The treating transplant physician determined target ranges of tacrolimus trough concentrations for their individual patient and changed the dosage if this was indicated. Tacrolimus exposure was assessed by means of venous limited sampling AUC (T=0, 1 and 3 hours). $AUC_{0\text{-}24h}$ calculation was done with pharmacokinetic modelling software (MwPharm 3.7,

MediWare, Prague). A dose adjusted $AUC_{0\text{-}24h}$ was calculated by dividing the $AUC_{0\text{-}24h}$ by the tacrolimus dosage in milligrams, assuming linearity between dosage and AUC. Dose adjusted $AUC_{0\text{-}24h}$ was used to quantify the DDI.

All subjects signed informed consent and the voluntariness of participation was underlined.

Results

5 pwCF completed the study (3 kidney and 2 liver transplants). During the study period, no serious adverse events were reported. Liver function tests remained within normal ranges in all subjects.

Dose adjusted $AUC_{0\text{-}24h}$ of tacrolimus

Dose adjusted $AUC_{0\text{-}24h}$ of all subjects are depicted in figure 1. In all subjects the dose adjusted $AUC_{0\text{-}24h}$ was higher at day 28 compared to day -14. Median (min-max) dose adjusted $AUC_{0\text{-}24h}$ at day 28 divided by day -14 was 1,79 (1,07-2,05).

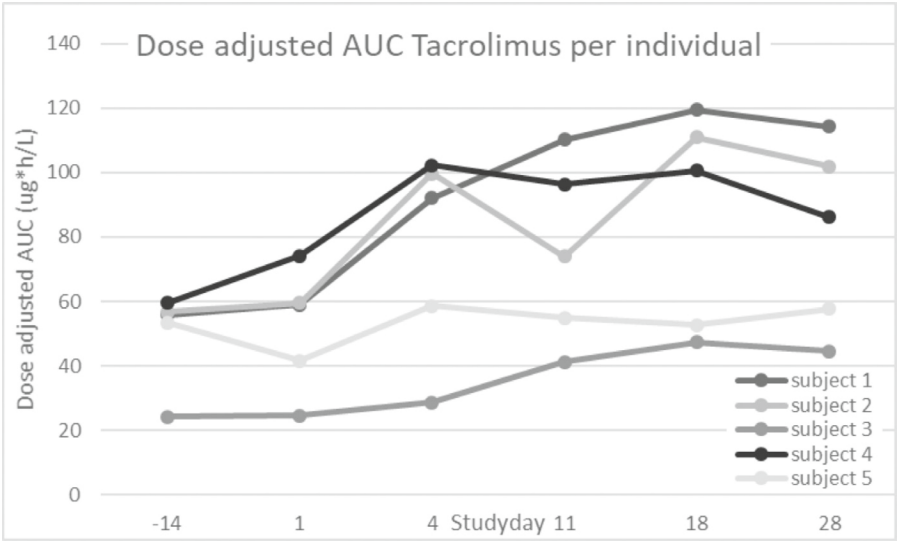

Figure 1. *dose adjusted $AUC_{0\text{-}24h}$ of tacrolimus.*

Concentration time profiles of tacrolimus

The concentration time profiles of tacrolimus obtained by the DBS method of all subjects are shown in figure 2. In individual 2, the tacrolimus dose was increased before the start of ETI to attain the target range. In 4 subjects, dose adjustments of tacrolimus during the ETI treatment period were performed. In individual 1, 2 and 3 dose reduction of tacrolimus was respectively 25%, 33,3% and 40%. In individual 5 the dose was increased by 60%.

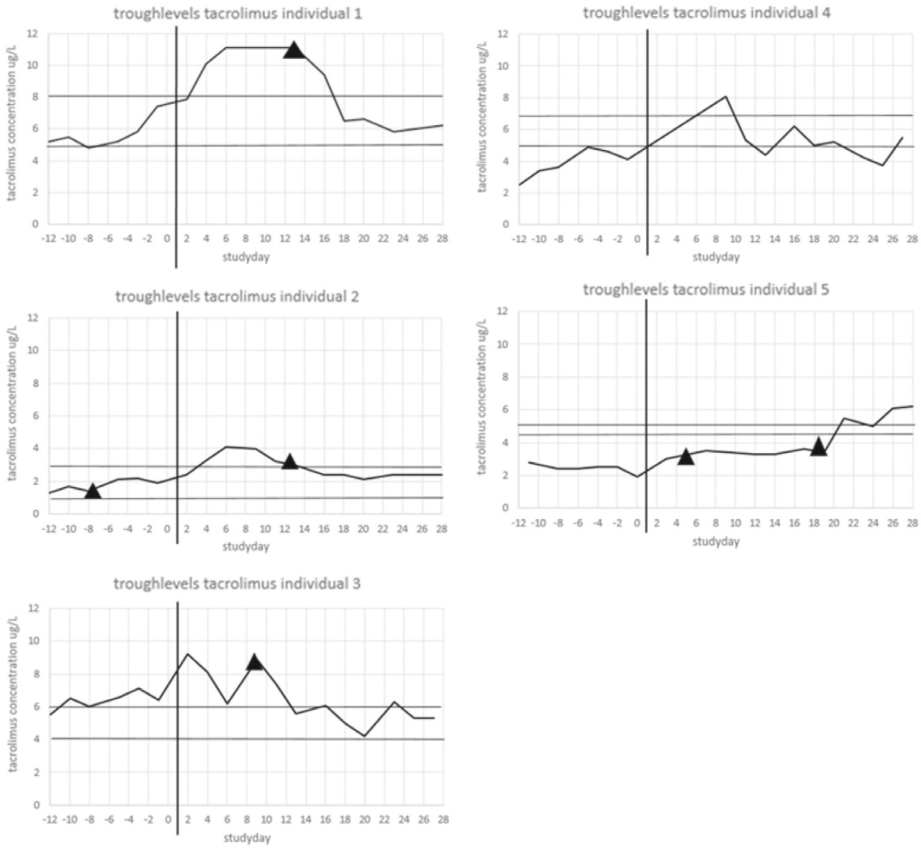

Figure 2. *Tacrolimus trough levels obtained by DBS sampling presented for each individual.*

The vertical line indicates the start of ETI, the 2 horizontal lines indicate the target range of tacrolimus as determined by the treating transplant physician, the triangles indicate each dose adjustment.

Clinical outcome and concentration time profiles of ivacaftor, tezacaftor and elexacftor

Median (min-max) increase in CFQ-R-respiratory domain (CFQ-R-RD) after one month of ETI treatment was 11,1 (0-22,2) points. Median (min-max) sweatchloride (SwCl) decrease was 55 (28-59) mmol/L. Median (min-max) FVC increase was 9 (4-16) percentage points and median (min-max) FEV_1 increase was 13 (6-16) percentage points.

The concentration time profiles of ivacaftor, tezacaftor and elexacaftor are shown per individual in figure 3. The median (min-max) trough concentration on day 28 of ivacaftor was 0,58 (0,38-1,23)µg/mL, of tezacaftor 1,63 (0,90-2,99)µg/mL and of elexacaftor 4,87 (3,64-10,26)µg/mL.

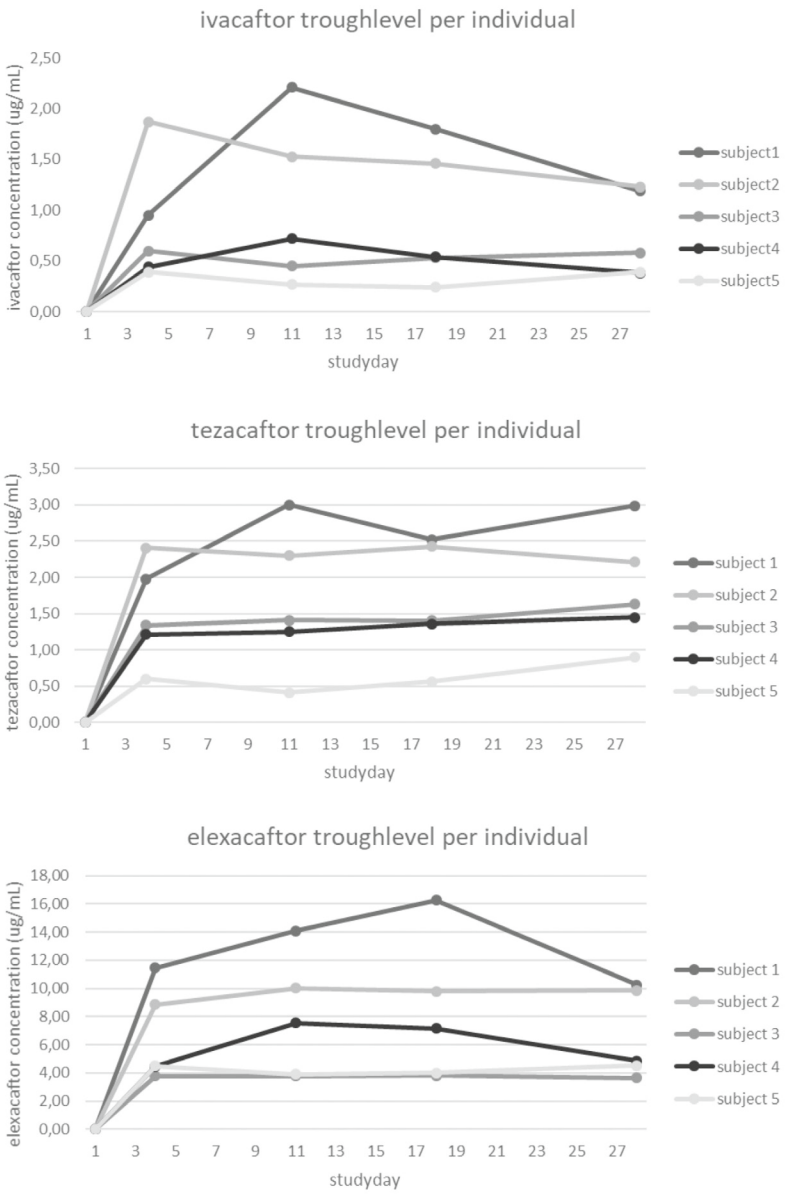

Figure 3. *Ivacaftor, tezacaftor and elexacaftor trough concentrations per individual, measured at day 1, 4, 11, 18 and 28.*

Discussion

The DDI between tacrolimus and ETI resulted in an increased exposure of tacrolimus in all subjects, the dose adjusted $AUC_{0\text{-}24h}$ was 1,79 times (median) higher at the end of the study. 5 dose adjustments were performed in 4 subjects in order to attain tacrolimus target range. No adverse events were reported and all subjects showed clinical improvement during ETI treatment.

To date, few case reports have been published describing their experience with ETI in liver transplanted pwCF [11-13]. These reports presented several patients with side effects mostly several months after initiation of ETI and therefore deemed to be related to ETI and not tacrolimus. We chose for a study duration of 4 weeks because we aimed to focus on side effects related to DDI. In Ragans' case series, tacrolimus trough concentrations were elevated in 7 patients (70%) [13]. In the case reports of McKinzie the only patient on tacrolimus treatment did not change tacrolimus dosing during ETI treatment [12]. These results are in line with our observations.

Although the number of subjects in our study is small, the clinical benefit of ETI treatment was evident in all subjects and comparable with the results of phase 3 studies in non transplanted pwCF [1, 2]. Trough concentrations of ivacaftor, tezacaftor and elexacaftor were within the range as mentioned in the SmPC of ETI [6] with a very high between-patient variability. This is in line with results of previous studies [14-15].

As far as we are aware, this is the first prospective DDI study on ETI treatment in kidney or liver transplanted pwCF using tacrolimus. By measuring limited sampling AUC's and trough levels of both tacrolimus and ETI we have obtained the best possible insight into the interaction of both drugs. The study conditions can be extrapolated and easily used in daily clinical practice. Although the duration of the study was relatively short, we showed that the DDI resulted in an elevated exposure to tacrolimus. We realize that our data have been influenced by the treating transplant physicians' decision to adjust the tacrolimus dose and the delay (3-4 days) between the DBS and their results. These circumstances however represent daily clinical practice. The lack of side effects reported in our study suggests that despite the small therapeutic window of tacrolimus, some degree of fluctuation in tacrolimus exposure is generally well tolerated.

Studies with a long term follow up are needed to gain better insight into the safety of co-administration of ETI and tacrolimus (e.g. hepatotoxicity, allograft function). Recently, several studies reported data of ETI use in lung transplanted pwCF [7, 10,

16-17]. Although the clinical benefit is less obvious, extrapulmonary manifestations may improve with ETI treatment [18-19]. To date, few studies found a high number of patients with side effects, most of them deemed to be related to ETI [16-17]. Larger studies are needed to get better insight in the risks and benefit in lung transplanted pwCF.

Based on the results of our study, we advise considering treatment with ETI in liver and kidney transplanted pwCF under the condition of close monitoring of tacrolimus levels and adverse events.

Acknowledgements

We thank all patients and their treating transplant physicians for their involvement in this study.

Authorship contribution statement

Renske van der Meer: methodology, formal analysis, investigation, resources, writing-original draft, writing-review&editing,visualization. **Erik B Wilms:** methodology, validation, formal analysis, writing-review&editing, visualization. **Margot N Eggermont:** investigation, resources, data curation, writing-review&editing. **Helena M Paalvast:** investigation, resources, data curation. **Richard C J M van Rossen:** validation, formal analysis, resources. **Harry G M Heijerman**: conceptualization, methodology, writing-review&editing, supervision.

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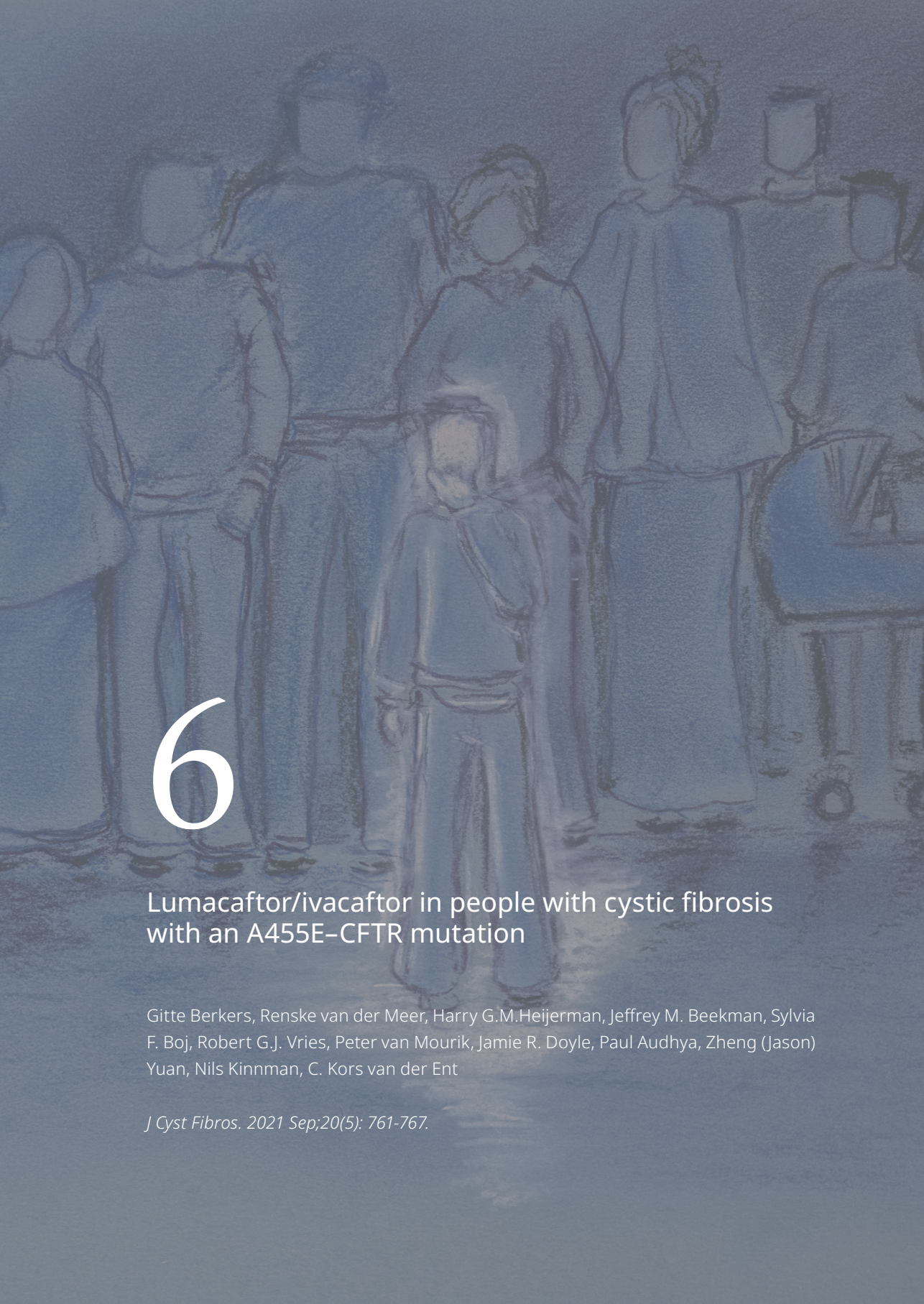

6

Lumacaftor/ivacaftor in people with cystic fibrosis with an A455E–CFTR mutation

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J Cyst Fibros. 2021 Sep;20(5): 761-767.

Abstract

Background: Previous in vitro organoid data showed A455E-CFTR, a rare CFTR mutation with 4.1% prevalence in the Netherlands, responds to lumacaftor/ivacaftor (LUM/IVA). We explored LUM/IVA's clinical efficacy in people with CF and ≥1 A455E-CFTR mutation.

Methods: Participants aged ≥12 years were randomized to 1 of 2 treatment sequences (LUM/IVA→placebo or placebo→LUM/IVA) with an 8-week washout period between. Primary endpoint was absolute change in ppFEV$_1$ from study baseline through 8 weeks. Additional endpoints were change in sweat chloride concentration (SwCl) and CFQ-R respiratory domain score. Correlations between organoid-based measurements and clinical endpoints were investigated.

Results: Twenty participants were randomized at 2 sites in the Netherlands. Mean absolute change in ppFEV$_1$ from study baseline through Week 8 showed a treatment difference of 0.1 percentage points (95% CI, -2.5 to 2.7; P = 0.928) between LUM/IVA (within-group mean change, 2.7) and placebo (within-group mean change, 2.6). The mean absolute change in SwCl concentration from study baseline through Week 8 showed a treatment difference of -7.8 mmol/L between LUM/IVA and placebo (P = 0.004), while the absolute change in CFQ-R respiratory domain score showed a treatment difference of 3.5 between LUM/IVA and placebo (P = 0.469). The in vitro organoid-based assay demonstrated a concentration-dependent swelling increase with LUM/IVA. Exploratory correlation analyses between organoid swelling and ppFEV$_1$ and SwCl outcomes showed correlation coefficients of 0.49 and -0.11, respectively.

Conclusions: In this exploratory study, LUM/IVA elicited an in vitro response in organoid swelling and in vivo response in SwCl in participants with CF and ≥1 A455E-CFTR mutation. The primary endpoint (ppFEV$_1$) did not show a statistically significant difference between LUM/IVA and placebo; correlations between in vitro and in vivo responses were not established.

Introduction

Cystic fibrosis (CF) results from mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene that reduce the quantity and/or function of the CFTR protein, which regulates chloride transport across epithelia in exocrine organs, including the lung and pancreas [1]. Progressive lung function decline is the leading cause of mortality among people with CF (pwCF) [2,3].

p.Ala455Glu (A455E) is a class V mutation that generates CFTR protein with a shortened half–life, resulting in a reduction of mature CFTR protein [4], [5], [6]; in vitro studies suggest that the quantity of functional protein at the cell surface is 12% of wild type [7]. With this amount of functional protein, A455E–CFTR is considered a residual function mutation. Worldwide, A455E mutations have been reported in <0.1% of pwCF, although the prevalence varies by region [8,9]; in the Netherlands, the A455E mutation occurs in 4.1% of pwCF [9,10]. Clinical experience with the A455E mutation, initially associated with a less–severe CF phenotype, has shown differences in disease severity by early adulthood, with a range of lung function loss [8,11,12]. Although people with residual function mutations such as A455E develop clinical characteristics of CF more slowly than those homozygous for F508del, it progresses more rapidly in adolescents and young adults [13].

Ivacaftor (IVA) is a small–molecule CFTR potentiator that increases the channel open probability of CFTR at the cell surface [14]. Lumacaftor (LUM) and tezacaftor are small–molecule CFTR correctors that increase the quantity of CFTR delivered to the cell surface; these are combined with a CFTR potentiator, such as IVA, for their additive effects [15,16]. IVA has been approved (as of 2017) in the United States for treating people with an A455E mutation [14], and the combination of IVA and tezacaftor has been approved (as of 2018) in the United States [16] and European Union [17]. In the European Union, combination IVA and tezacaftor treatment is indicated for pwCF with an A455E–CFTR mutation who also have an F508del–CFTR mutation [17]. Combined lumacaftor/ivacaftor (LUM/IVA) therapy improves lung function and provides multisystemic clinical benefits in pwCF who are homozygous for the F508del mutation, a mutation that results in processing and trafficking defects [18]. Improvements in forced expiratory volume in 1 s (FEV1) were observed as early as Day 15 in participants ≥12 years of age on LUM/IVA compared with those on placebo and were sustained through 24 weeks of treatment in the pivotal Phase 3 studies TRAFFIC and TRANSPORT [18]. Additional studies of LUM/IVA have led to approval of its use in pwCF as young as 2 years who are homozygous for the F508del mutation [15,19].

In vitro responses to CFTR modulators have previously been studied using Fischer rat thyroid or human bronchial epithelial cell systems [20,21]. A study in human bronchial epithelial cultures from pwCF homozygous for the F508del mutation showed that LUM enhanced forskolin–stimulated chloride and fluid transport; the addition of IVA increased this response [22]. More recently, a novel CFTR functional assay using cultures of intestinal stem cells, referred to as organoids, was developed [23]. Briefly, organoids derived from the intestinal stem cells of healthy controls swell in response to forskolin–induced activation of CFTR–dependent chloride secretion. Forskolin–induced swelling (FIS) is reduced in organoids derived from pwCF homozygous for the F508del mutation compared with those from healthy controls and could be restored by incubation of the organoids with LUM/IVA. LUM/IVA–induced improvement of organoid swelling was also observed in A455E/F508del organoids [24]. These in vitro data suggest that correction and potentiation by LUM/IVA may improve CFTR function in people with A455E–CFTR mutations.

Based on these preclinical data, we designed this study to explore the efficacy and in vitro responses of LUM/IVA in pwCF who had ≥1 A455E–CFTR mutation.

Methods

Clinical study design and participants

This exploratory, randomized, double–blind, placebo–controlled, multicenter, Phase 2 crossover study took place in the Netherlands (VX15–809–111; NCT03061331). It included two 8–week treatment periods (±7 days) separated by an 8–week (±7 days) washout period (Fig. 1A). Treatment Period 1 was from Day 1 to Week 8, and Treatment Period 2 was from Week 16 to Week 24. Participants were randomized 1:1 to receive the 2 treatment sequences. In Treatment Sequence 1, participants received LUM/IVA in Treatment Period 1 and placebo in Treatment Period 2 (LUM/IVA→P). In Treatment Sequence 2, participants received placebo in Treatment Period 1 and LUM/IVA in Treatment Period 2 (P→LUM/IVA). The approved dose of LUM/IVA (LUM 400 mg/IVA 250 mg every 12 h [q12h]) or matching placebo q12h was given orally. An 8–week washout period between the 2 treatment periods was chosen based on the terminal half–lives of LUM (26 h) and IVA (12 h) and on previous clinical study results [14,15,19].

Given the limited participant population available, a crossover design was chosen that enabled treatment of the same participant with both placebo and LUM/IVA in different treatment periods. The use of a double–blind design reduced the chance of bias. Participants with stable CF who were ≥12 years of age with ≥1 A455E–CFTR mutation

and a percent predicted FEV_1 s ($ppFEV_1$) of ≥30% and ≤90% were eligible. This study was conducted in accordance with the International Council for Harmonisation Good Clinical Practice (ICH GCP) guidelines, consistent with the principles of the Declaration of Helsinki. Study documentation was approved by institutional ethics committees for each study site. All participants (and/or their legal guardians) provided written informed consent.

Objective and outcomes

Clinical and in vitro responses to LUM/IVA in participants ≥12 years of age with CF with the A455E–CFTR mutation were investigated. The primary endpoint was absolute change in $ppFEV_1$ from study baseline through 8 weeks of treatment of either treatment period, calculated using the 2012 Global Lung Initiative equations [25]. Other endpoints included absolute change in sweat chloride concentration from study baseline through 8 weeks of treatment and absolute change in Cystic Fibrosis Questionnaire–Revised (CFQ–R) respiratory domain score from study baseline at the end of 8 weeks of treatment in either period.

All treatment–emergent adverse events (AEs; defined as AEs that increased in severity or were newly developed at or after the initial dose of study drug in a given treatment period to 28 days after the last dose of study drug in that treatment period [or safety follow–up visit, whichever was last]) were assessed, documented, and reported in accordance with ICH GCP guidelines.

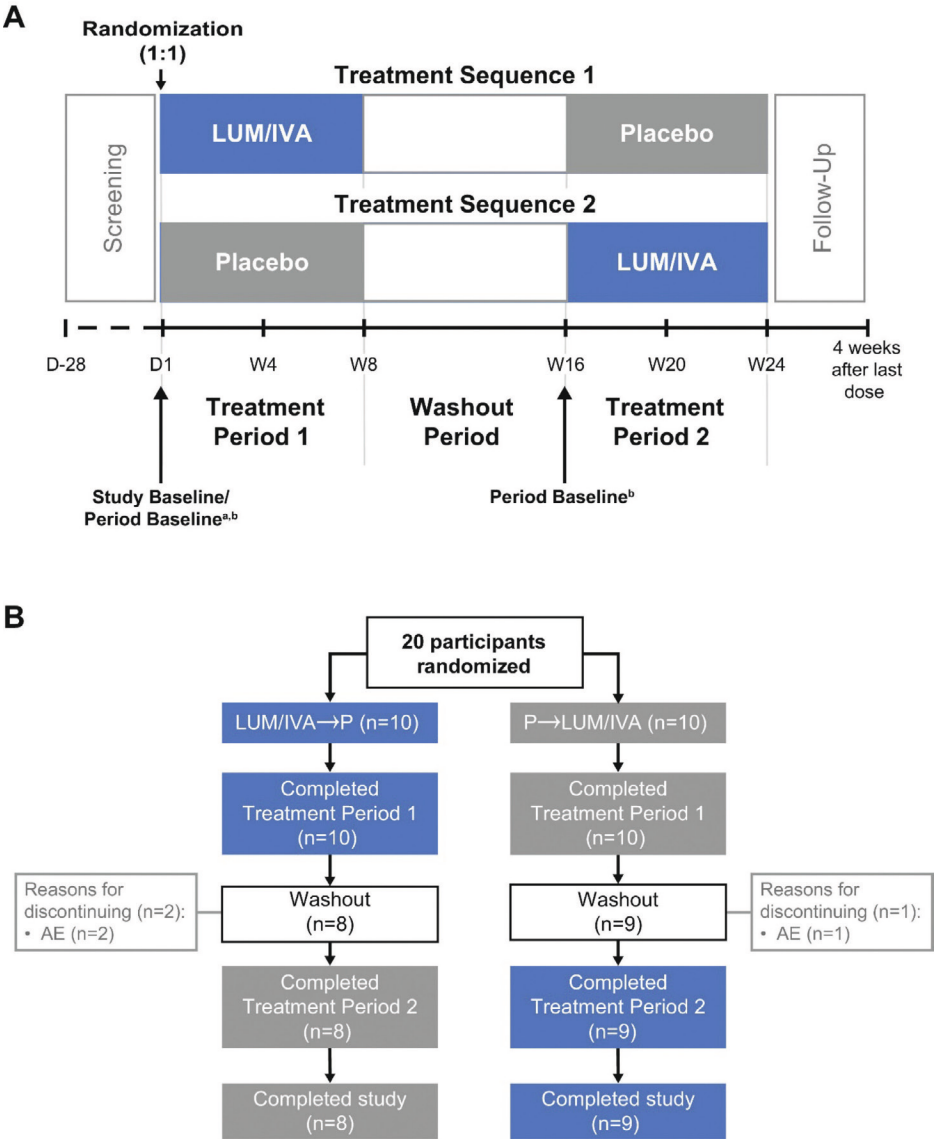


Figure 1. *Study Design and Participant Disposition.*

***A**. In this Phase 2, double–blind, placebo–controlled, crossover study, eligible participants were randomized (1:1) to 1 of 2 treatment sequences (LUM/IVA followed by placebo [Treatment Sequence 1] or placebo followed by LUM/IVA [Treatment Sequence 2]), consisting of two 8–week treatment periods separated by an 8–week washout period.*

***B.** Overall, 20 participants were randomized; all received ≥1 dose of study drug and completed Treatment Period 1. Of 10 participants randomized to Treatment Sequence 1, eight completed both treatment periods, and two discontinued treatment during the washout period due to AEs. Of 10 participants randomized to treatment sequence 2, nine completed both treatment periods, and*

one discontinued treatment during the washout period due to AEs. AE, adverse event; D, day; IVA, ivacaftor; LUM, lumacaftor; P, placebo; W, week. a Study baseline was the most recent nonmissing measurement (scheduled or unscheduled) collected prior to the first dose of study drug (either placebo or LUM/IVA) in the study. b Period baseline was the most recent nonmissing measurement (scheduled or unscheduled) collected before the first dose of study drug in Treatment Period 1 or Treatment Period 2.

Statistical analysis

Because the A455E–CFTR mutation is so rare, no formal sample size calculations were conducted for this exploratory study. The planned sample size of 20 participants was based on the number of pwCF expected to be available and willing to participate. Assuming an estimated SD of the paired differences of 8.00 in ppFEV1, the available sample size of 20 participants produces a 2–sided 95% CI for the mean treatment difference, with a precision (margin of error) of 3.74 percentage points.

For this crossover study, 2 different baselines were defined (Fig. 1A). Study baseline was defined as the most recent nonmissing measurement (scheduled or unscheduled) collected prior to the first dose of study drug (either placebo or LUM/IVA) in the study. The definition was applied to all demographics, background, and baseline characteristics and also to data analyses, including the primary endpoint analysis. Period baseline was defined as the most recent nonmissing measurement (scheduled or unscheduled) collected before the first dose of study drug in Treatment Period 1 or Treatment Period 2. Absolute changes from study baseline and period baseline were calculated as the postbaseline value minus the study baseline and period baseline value, respectively.

The primary analysis for the primary efficacy endpoint, the absolute change in ppFEV$_1$ from study baseline through 8 weeks of treatment of either treatment period, was based on a mixed–effects model for repeated measures (MMRM). The model included the absolute change from the study baseline in each treatment period as the dependent variable, with sequence, treatment, period, visit within period, and treatment–by–visit interaction as fixed effects; study baseline ppFEV$_1$ as a covariate; and participant nested within sequence as the random effect. In the model, visit was treated as a class variable. An unstructured covariance matrix was assumed for the repeated measurements of the same participant within each treatment period. Similar analyses were done for the other endpoints (sweat chloride concentration and CFQ–R respiratory domain score), with the baseline of the analyzed endpoint as the covariate. Differences between LUM/IVA and placebo endpoints through 8 weeks of treatment were obtained from the MMRM models, estimated by least–squares mean with a 2–sided 95% CI and a 2–sided P value. All reported P values for other endpoints are

nominal P values. There was no control for multiplicity for this exploratory study. As a supportive sensitivity analysis, a prespecified MMRM analysis was conducted for the changes from period baseline in the primary endpoint $ppFEV_1$.

Participant–derived organoid–based measurements (FIS assay)

Participant–derived organoid responses to LUM/IVA and correlations to clinical outcomes ($ppFEV_1$, sweat chloride concentration) were also explored. Rectal biopsies were performed for individual participants during screening, and specimens were shipped to Hubrecht Organoid Technology, where intestinal crypts were isolated and expanded to establish organoid cultures. Organoid swelling was measured with an FIS assay using 42 different experimental conditions.

The background–corrected area under the curve (AUC) of organoid swelling at each experimental condition was summarized descriptively. Background–corrected swelling value refers to the difference between swelling of any nonzero LUM/IVA condition and that of the corresponding zero LUM/IVA condition at the same forskolin concentration. An exploratory correlation analysis between the in vitro organoid–based measurements and the responses to LUM/IVA treatment from period baseline in $ppFEV_1$ and sweat chloride concentration was conducted. The experimental conditions selected for the correlation analyses (forskolin, 0.128 μM; LUM, 3 μM; IVA, 3 μM) showed a large differentiation to forskolin alone and have previously shown correlation of organoid swelling response with a population–level clinical response [24]. Given the small sample size, Spearman rank correlation was used in the correlation analysis.

Results

Twenty participants were randomized 1:1 to receive the 2 treatment sequences at the 2 study sites. After randomization, participants continued their concomitant medications, most commonly for CF management (e.g., salbutamol, dornase alfa, and azithromycin).

Overall, 60% of participants were female, and the mean age was 38 years, with the majority (90%) being ≥18 years of age (Table 1). Ninety percent (18 of 20) of participants had an F508del–CFTR mutation on the second allele; the rest had E60X–CFTR on the second allele. Overall, the mean $ppFEV_1$ was 58.9 percentage points (range, 31.3 to 94.9) at baseline. All 20 randomized participants received ≥1 dose of study drug and were included in both the full analysis set and the safety set. All participants completed the 8 weeks of dosing in Treatment Period 1, and 17 (85%) completed the

8 weeks of dosing in Treatment Period 2 (Fig. 1B). Three participants discontinued the study during the washout period due to AEs. All 3 AEs were infective pulmonary exacerbations of CF that occurred outside the treatment–emergent period, were mild or moderate in severity, and deemed unrelated to the study drug. No participant discontinued during either treatment period.

Table 1. *Baseline participant demographics and characteristics.*

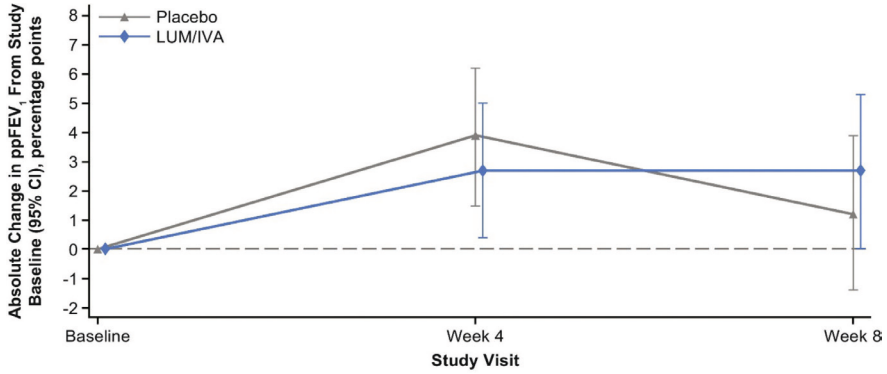
	LUM/IVA→P (n = 10)	P→LUM/IVA (n = 10)	Overall (N = 20)
Female, n (%)	7 (70.0)	5 (50.0)	12 (60.0)
Age, mean (range), years	41.2 (14–-59)	34.7 (18–-51)	38.0 (14–-59)
≥12 years to <18 years, n (%)	2 (20.0)	0	2 (10.0)
≥18 years, n (%)	8 (80.0)	10 (100.0)	18 (90.0)
White, n (%)	10 (100.0)	10 (100.0)	20 (100.0)
Mutation genotype, n (%)			
A455E/F508del	9 (90.0)	9 (90.0)	18 (90.0)
A455E/other[a]	1 (10.0)	1 (10.0)	2 (10.0)
Weight, mean (SD), kg	64.8 (12.8)	72.6 (11.5)	68.7 (12.5)
Height, mean (SD), cm	170.3 (6.9)	178.2 (8.4)	174.3 (8.5)
BMI, mean (SD), kg/m^2	22.3 (3.5)	22.9 (3.5)	22.6 (3.4)
ppFEV$_1$, n (%)			
<40%	0	2 (20.0)	2 (10.0)
≥40% to <70%	9 (90.0)	5 (50.0)	14 (70.0)
≥70% to ≤90%	1 (10.0)	2 (20.0)	3 (15.0)
>90%	0	1 (10.0)	1 (5.0)
Mean ppFEV$_1$ (SD), percentage points	57.7 (9.4)	60.0 (20.0)	58.9 (15.3)
Mean sweat chloride concentration (SD), mmol/L	77.2 (12.3)	82.5 (7.5)	79.8 (10.3)
Mean CFQ–R respiratory domain score (SD)[b]	69.4 (15.3)	67.8 (13.8)	68.6 (14.2)
History of pancreatic insufficiency, n (%)	2 (20.0)	1 (10.0)	3 (15.0)

BMI, body mass index; CFQ–R, Cystic Fibrosis Questionnaire–Revised; LUM/IVA→P, participants receiving lumacaftor/ivacaftor in Treatment Period 1 followed by placebo in Treatment Period 2; P→LUM/IVA, participants receiving placebo in Treatment Period 1 followed by LUM/IVA in Treatment Period 2; ppFEV$_1$, percent predicted forced expiratory volume in 1 s.

a. *The 2 participants in the "other" mutation group had a class I E60X* mutation.
b. *Data from the CFQ–R "Ages 12 and 13" and "Adolescents and Adults" versions were pooled for analysis.*

The estimated mean absolute change in ppFEV$_1$ from study baseline through 8 weeks of treatment (primary endpoint) showed a treatment difference of 0.1 percentage points (95% CI, –2.5 to 2.7; P = 0.928) between LUM/IVA and placebo (least–squares absolute mean change: LUM/IVA, 2.7 percentage points [SE, 1.1]; placebo, 2.6 percentage points [SE, 1.2]; Fig. 2). In the prespecified supportive analysis, the estimated mean within–group absolute change in ppFEV$_1$ from period baseline through 8 weeks was 3.2 percentage points (SE, 1.0) with LUM/IVA and 1.1 percentage points (SE, 1.0) with placebo, which resulted in a treatment difference of 2.1 percentage points (95% CI, –0.6 to 4.8; P = 0.117; Table 2). The change in ppFEV$_1$ from baseline was further assessed for

both Treatment Period 1, which was not subject to the impact of treatment crossover, and Treatment Period 2.



	Placebo (n=18)	LUM/IVA (n=19)
Baseline ppFEV$_1$, mean (SD), percentage points	59.4 (15.9)	57.6 (14.6)
Absolute change in ppFEV$_1$ through Week 8, LS mean (95% CI), percentage points	2.6 (0.2 to 4.9)	2.7 (0.3 to 5.0)
P value within treatment	0.034	0.027
Difference, LS mean (95% CI), percentage points	0.1 (-2.5 to 2.7)	
P value vs placebo	0.928	

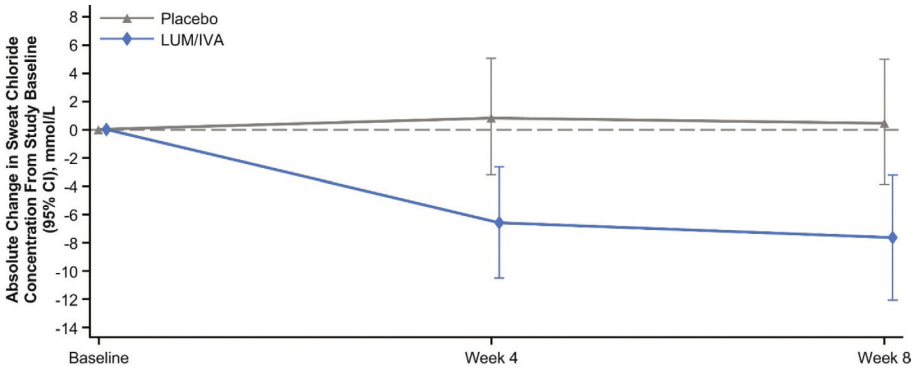
Figure 2. *Absolute Change in ppFEV$_1$ From Study Baseline Through Week 8 of Treatment. All participants received LUM 400 mg/IVA 250 mg every 12 h (blue line/diamonds) for 8 weeks and placebo (gray line/triangles) for 8 weeks according to 1 of 2 treatment sequences (LUM/IVA→placebo or placebo→LUM/IVA) with an 8–week washout period. Absolute change is expressed as LS mean (95% CI). IVA, ivacaftor; LS, least squares; LUM, lumacaftor; ppFEV1, percent predicted forced expiratory volume in 1 s.*

Table 2. *Absolute change from period baseline in ppFEV1 through Week 8.*

	Placebo (n = 18)	LUM/IVA (n = 19)
Period baseline ppFEV$_1$, mean (SD), percentage points	60.6 (15.6)	56.6 (13.7)
Absolute change from period baseline through Week 8		
LS mean (95% CI)	1.1 (−1.0 to 3.3)	3.2 (1.1 to 5.4)
P value within treatment	0.291	0.005
LS mean difference (95% CI)	2.1 (−0.6 to 4.8)	
P value vs placebo	0.117	

IVA, ivacaftor; LS, least squares; LUM, lumacaftor; ppFEV$_1$, percent predicted forced expiratory volume in 1 s.

The mean absolute change in sweat chloride concentration from study baseline through Week 8 showed a treatment difference of –7.8 mmol/L (95% CI, –12.6 to –3.1; nominal P = 0.004) between the LUM/IVA group and the placebo group (Fig. 3). Changes of –7.1 mmol/L (SE, 1.7) in the LUM/IVA group and 0.7 mmol/L (SE, 1.8) in the placebo group were observed.



	Placebo (n=18)	LUM/IVA (n=19)
Baseline sweat chloride concentration, mean (SD), mmol/L	81.6 (9.1)	80.1 (10.5)
Absolute change in sweat chloride concentration through Week 8, LS mean (95% CI), mmol/L	0.7 (-3.0 to 4.4)	-7.1 (-10.7 to -3.5)
P value within treatment	0.688	0.0004
Difference, LS mean (95% CI), mmol/L	-7.8 (-12.6 to -3.1)	
P value vs placebo	0.004	

Figure 3. *Absolute change from Study Baseline in sweat chloride concentration through Week 8 of treatment. All participants received LUM 400 mg/IVA 250 mg every 12 h (blue line/diamonds) or placebo (gray line//triangles) for 8 weeks according to 1 of 2 treatment sequences (LUM/IVA→placebo or placebo→LUM/IVA). Absolute change is expressed as LS mean (95% CI). IVA, ivacaftor; LS, least squares; LUM, lumacaftor.*

The mean absolute change in CFQ–R respiratory domain score from study baseline to the end of week 8 showed a treatment difference of 3.5 points (95% CI, –6.4 to 13.4; nominal P = 0.469) between the LUM/IVA group and the placebo group. Changes of 6.4 points (SE, 3.9) in the LUM/IVA group and 2.9 points (SE, 4.0) in the placebo group were observed.

Administration of LUM/IVA in this CF population for approximately 8 weeks was generally safe and well tolerated. No participants had serious AEs or AEs that led to

treatment discontinuation or interruption during the treatment period. The safety results were consistent with the known safety profile of LUM/IVA [18,26].

Of the 20 participants enrolled in the study, organoid cultures were successfully established for 16: Fourteen participants with the A455E/F508del genotype and 2 participants with the A455E/E60X genotype had organoid data.

The results of the in vitro organoid–based assay demonstrated a concentration–dependent increase in background–corrected AUC of swelling with LUM/IVA treatment. The background–corrected swelling response (i.e., AUC) was maximal and best differentiated at the forskolin 0.128–μM concentration and saturated at or above the LUM 3 μM/IVA 3 μM concentrations. At this selected condition (forskolin 0.128 μM and LUM 3 μM/IVA 3 μM), the Spearman rank correlation coefficients between organoid AUC and the changes in $ppFEV_1$ and sweat chloride concentration observed with LUM/IVA treatment were 0.49 (n = 14; P = 0.078) and –0.11 (n = 15; P = 0.685), respectively.

Discussion

Demonstrating the clinical efficacy of novel therapies targeting rare mutations or small participant populations is challenging. This exploratory study was conducted in a small cohort of pwCF with ≥1 A455E–CFTR mutation to evaluate the impact of LUM/IVA on clinical and in vitro endpoints.

The primary endpoint, absolute change in $ppFEV_1$ from study baseline through 8 weeks of treatment, did not show a significant treatment difference between the placebo and LUM/IVA groups. During this study, 2 participants had substantial increases in $ppFEV_1$ after 8 weeks of LUM/IVA treatment in Treatment Period 1, but their $ppFEV_1$ values did not return to study baseline level after the 8–week washout period. Given the study's small sample size, estimation of treatment effect based on the changes from study baseline can be impacted substantially by these 2 outlier participants due to the underlying assumption of equal baselines for Treatment Period 1 and Treatment Period 2. The prespecified supportive analysis of the changes in $ppFEV_1$ from period baseline does not depend on this assumption and showed a treatment difference of 2.1 percentage points between LUM/IVA and placebo.

Although the study failed to meet the primary endpoint, it is important to note that a treatment difference was observed between LUM/IVA and placebo in sweat chloride concentration. The overall efficacy results were suggestive of a clinical response

with LUM/IVA treatment in pwCF with ≥1 A455E mutation. Potential long–term benefits, such as changes in the rates of pulmonary exacerbations, FEV_1 decline, and hospitalizations, were not evaluated in this study.

The rectal organoid FIS assay can be an effective strategy to identify rare CFTR mutations for CFTR modulator precision medicines. In the current study, a clear, concentration–dependent, in vitro organoid response to LUM/IVA was observed with participant–derived organoids, further suggesting that pwCF with the A455E mutation could be responsive to LUM/IVA.

Previous studies demonstrated that organoid swelling correlated with clinical changes in $ppFEV_1$ when participant outcomes were pooled from a heterogenous population and compared with preclinical in vitro results from different participants [24]. Moreover, Berkers et al recently published an analysis correlating in vitro organoid measurements with in vivo response of sweat chloride concentration and $ppFEV_1$ [27], their results suggested that the organoid outcome was predictive of clinical outcome in individual participants. However, the current study could not demonstrate conclusive evidence regarding a correlation between the swelling of organoids and $ppFEV_1$ or sweat chloride response in pwCF with an A455E–CFTR mutation. The homogenous population of participants in this small study and the relatively small effects observed could have contributed to the results seen in this study.

Administration of LUM 400 mg/IVA 250 mg q12h for up to 8 weeks was safe and well tolerated in pwCF with the A455E–CFTR mutation. Safety results were consistent with those seen in other trials, and no new unexpected AEs were identified.

Conclusion

In this exploratory study, an in vitro response to LUM/IVA was observed in participant–derived organoids, and improvements in SwCl concentration were observed in pwCF treated with LUM/IVA compared to placebo. However, the primary clinical endpoint of absolute change in $ppFEV_1$ did not show a statistically significant difference between LUM/IVA and placebo, and correlations between in vitro and in vivo responses were not established.

Authorship contribution statement

Gitte Berkers: Conceptualization, Methodology, Investigation, Resources, Writing - review & editing, Project administration. **Renske van der Meer:** Conceptualization, Methodology, Investigation, Writing - review & editing, Supervision. **Harry Heijerman:** Conceptualization, Methodology, Resources, Writing - original draft, Writing - review & editing, Visualization. **Jeffrey M. Beekman:** Conceptualization, Methodology, Writing - review & editing. **Sylvia F. Boj:** Supervision, Project administration. **Robert G.J. Vries:** Conceptualization, Methodology, Writing - review & editing, Supervision. **Peter van Mourik:** Investigation, Resources, Writing - review & editing. **Jamie R. Doyle:** Conceptualization, Methodology, Writing - original draft, Writing - review & editing. **Paul Audhya:** Conceptualization, Methodology, Formal analysis, Investigation, Resources, Writing - original draft, Writing - review & editing, Visualization, Supervision, Project administration, Funding acquisition. **Zheng (Jason) Yuan:** Methodology, Formal analysis, Writing - review & editing. **Nils Kinnman:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing - original draft, Writing - review & editing, Visualization, Supervision, Project administration. **C. Kors van der Ent:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Writing - review & editing, Visualization, Supervision, Funding acquisition.

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7

Breast development in a 7 year old girl with CF treated with ivacaftor: An indication for personalized dosing?

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Abstract

Substantial progress has been made in the treatment of Cystic fibrosis due to introduction of CFTR modulators. However, little is known about the long term side effects of treatment with these drugs. We here present a 7 year old girl with CF who presented with breast development as a rare dose dependent side effect of treatment with ivacaftor and we report data on the correlation between drug plasma concentration and clinical effect, bodyweight, and BSA in 16 patients. Higher plasma concentrations did not correlate with clinical effect, as change in FEV_1 and sweat chloride concentration. Patients with low bodyweight or BSA tended to have higher plasma concentrations. This might indicate that the current recommended dose of ivacaftor is at the top of the dose-response curve and that some patients can be treated with lower doses of ivacaftor with similar clinical effect.

Introduction

Treatment of patients with Cystic Fibrosis (CF) has been challenging for decades, but Cystic Fibrosis Transmembrane conductance Regulator (CFTR) modulators such as ivacaftor, lumacaftor, tezacaftor and lately elexacaftor impressively changed the perspectives [1, 2]. Initially, treatment with CFTR modulators started in adults and children aged 12 years and older, but treatment is now becoming available to younger children from the age of 6 months [3–5]. Ivacaftor, a CFTR-potentiator, is prescribed in adults and children aged 6 years and older with a body weight above 25 kg in a dose of 300 mg /day (mg/day). Little research is available on the optimal dose for younger children. Davies et al. [3] and Rosenfeld et al. [4] treated children aged 2–5 years and 1,2 years, respectively, with 100–150 mg/day. No data is available on the effect of lower doses of ivacaftor in these young children.

Extensive research into the short term effects and safety of treatment with ivacaftor shows positive results [3, 4, 6]. However, the safety on the long term remains unclear. Here we report a rare side effect of treatment with ivacaftor in a pediatric patient, which appears to be related to the prescribed dose of ivacaftor. Additionally, we report data on the correlation between plasma concentrations of ivacaftor and body weight, body surface area (BSA) and clinical effect.

Case presentation

A female CF patient aged 7 & 5/12th years old, who harbored the 711 + 1G > T and S1251N mutation, was presented to the outpatient clinic with breast development. She was being treated with ivacaftor for 3 years. At presentation, she was being treated with ivacaftor 300 mg/day, i.e. 10 mg/kg/day. The girl was in a stable condition, was pancreas sufficient, and had a normal FEV_1 (121% of the predicted value) and sweat chloride concentration (SCC) was 22 mmol/L (pre-treatment 91 mmol/L). At physical examination she had clearly visible breast development (Tanner stadium III-IV), without any other symptoms of pubertas praecox. Additional work- up showed a slightly elevated Luteinizing Hormone (LH) 2,1 U/L (reference range prepubertal girls < 1,0 U/L) and normal Follicle Stimulating Hormone (FSH) 2,9 U/L (reference range prepubertal girls 3–10 U/L) and estradiol < 40 pmol/L (reference range pre-pubertal girls < 60 pmol/L). Bone age assessment was performed, which was in accordance with her calendar age. An ultrasound of the breast showed symmetric development of glandular breast tissue without other abnormalities. As breast disorders are a known side effect of ivacaftor, the dose was reduced and later treatment was discontinued. Cessation of treatment led to quick total regression of the breast development to Tanner stadium I. Several weeks after discontinuation, she presented with increased

symptoms of dyspnea, productive cough, and reduced physical functioning. A decline of FEV_1 (112% of predicted value) was observed, SCC was not reevaluated. Therefore, treatment with low-dose ivacaftor (25% of original dose, i.e. 75 mg/day, 2.5 mg/kg/day) was restarted. The symptoms improved significantly within several weeks, lung function restored to earlier values (FEV_1 120% of predicted value) and SCC at this dose was 6 mmol/L. As clinical symptoms improved significantly and the SCC decreased to normal levels, there was no reason to increase the dose of ivacaftor, so a dose of 75 mg/day was continued. No breast development was observed until now after being treated with this dose for 17 months. In this case the appearance of premature telarche seems to be related to the dose of ivacaftor. Based on these findings we studied the relation between the plasma concentration of ivacaftor and a patient's weight and clinical effect to treatment, in a group of patients that was treated with ivacaftor.

Patients and methods

As part of an investigator initiated clinical trial (Berkers et al. [7]), we studied the plasma levels of ivacaftor in samples from 16 patients, who were treated with ivacaftor 150 mg twice daily. After 8 weeks of treatment, change from baseline in lung function (FEV_1% predicted) and SCC were measured as well as plasma ivacaftor levels. All blood samples were taken 4 h after dosing. Ivacaftor levels were measured with high-performance liquid chromatography (HPLC). Plasma levels were correlated to patient body weight and change in clinical parameters (FEV_1 and SCC) using the Pearson correlation coefficient.

Results

An overview of patient characteristics are shown in Table 1. The mean age of the patients was 21 (6–44) years and mean body weight was 55.4 (19.8- 95,8) kilograms. Only three patients were pancreas sufficient. Nine patients were treated with drugs that inhibit cytochrome 450 (CYP)3A4. One patient was treated with a strong inhibitor and 8 patients with weak inhibitors [8, 9]. An full overview of co-medication prescribed during the trial can be found in Supplemental table 1 in the appendix. After 8 weeks of treatment mean FEV1 improved 12,5%, mean SCC decreased 54,5 mmol/L. The mean post-dosing plasma level of ivacaftor was 5,03 umol/L. We found no significant correlation between the plasma concentrations of ivacaftor and changes in FEV_1 (Fig 1 A) or SCC (Fig 1 B). Patients with low body weight or low body surface area tended to have higher plasma concentrations of ivacaftor (Fig. 1 C and 1 D respectively).

Especially, patients with a body weight of approximately 20 kg demonstrated higher plasma concentrations compared to the other patients.

Table 1. *baseline characteristics of patients.*

	Mutation	Age (years)	Sex	Weight (kg)	Body surface area (m^2)	FEV1 (%)	SCC (µmol/L)	Pancreatic insufficiency	Use of CYP3A4 inhibitors
1	S1251N/ R117H	41	male	95.8	2.18	83	45	no	azithromycin, omeprazole
2	S1251N/ delta F508	44	female	71.4	1.85	73	41	yes	omeprazole
3	S1251N/ 1717-IGA	38	male	57.2	1.68	28	72	yes	azithromycin
4	S1251N/ delta F508	6	male	19.8	0.81	82	146	yes	none
5	S1251N/ delta F508	35	male	76.5	1.98	56	117	yes	azithromycin, esomeprazole
6	S1251N/ delta F508	32	female	59.5	1.68	65	110	yes	azithromycin
7	S1251N/ delta F508	9	male	30.5	1.11	73	64	yes	itraconazol, azithromycin
8	S1251N/ delta F508	13	female	42.1	1.34	63	70	yes	none
9	S1251N/ delta F508	17	female	67.8	1.81	73	63	yes	azithromycin
10	S1251N/ delta F508	15	female	49.1	1.48	66	67	no	none
11	S1251N/ delta F508	12	male	44.2	1.39	72	80	yes	none
12	S1251N/ A455E	16	male	68.2	1.83	95	86	no	none
13	S1251N/ delta F508	9	male	29.5	1.06	109	109	yes	none
14	S1251N/ delta F508	26	male	82.0	2.05	101	115	yes	azithromycin, omeprazole
15	S1251N/ delta F508	16	male	69.5	1.89	87	76	yes	azithromycin
16	S1251N/ R117H	6	male	23.0	0.9	97	21	yes	none
	Mean	21		55.4	1.56	76	80		

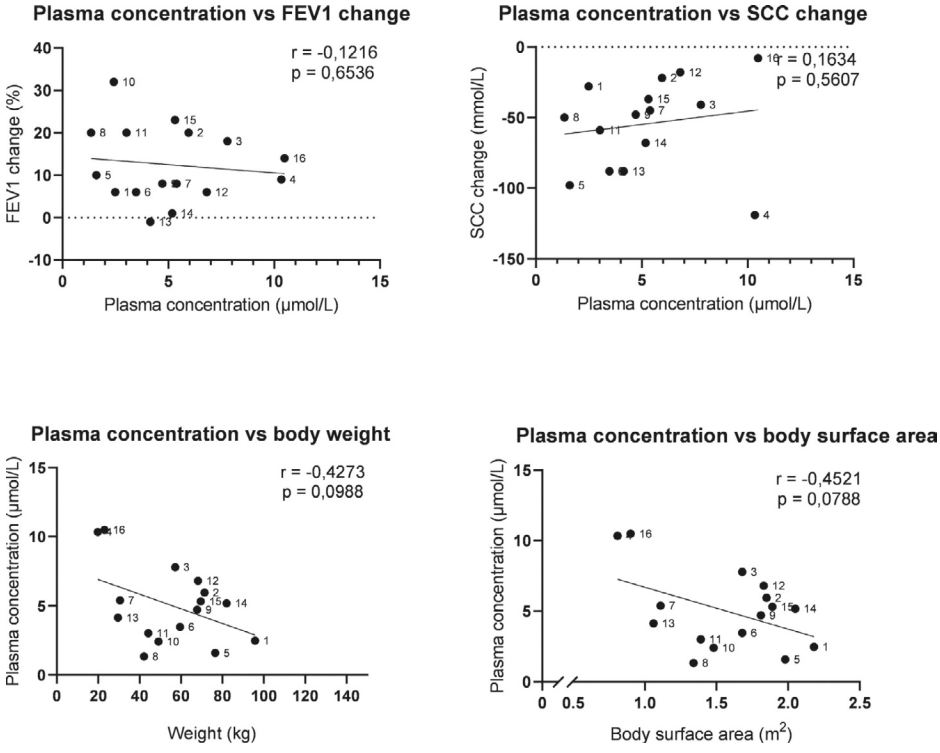

Figure 1. *Correlation between plasma concentration and change in FEV1 and SCC, body weight and BSA.*

Discussion

We describe breast development in a young girl during treatment with ivacaftor, which disappeared after cessation of treatment and did not reappear after resuming treatment with 25% of the recommended dose. Her clinical response was comparable to treatment with the full dose. This raises the question whether the currently advised dosage of ivacaftor may be too high and can thereby lead to side effects, such as premature breast development. Accurso et al. [6] reported that the incidence of adverse events was lowest in patients treated with 50 mg/day. The incidence of adverse events was similar in patients treated with 150, 300 or 500 mg/day. Frequently reported adverse events included cough, pulmonary exacerbations, erythema, diarrhea, abdominal pain and vomiting. However, these results should be interpreted with caution considering the small sample size within these groups and most of the adverse events are inherent to the disease cystic fibrosis. Breast development in children under 12 years old has not yet been reported as a side effect of ivacaftor. However, other breast disorders such as breast mass, breast swelling and gynecomastia have been described in both male and female patients aged 12 years and older [10, 11]. In this case the appearance of premature breast development seems to be related to the dose of ivacaftor as sex hormone levels were normal and total regression was observed after cessation of ivacaftor. Nevertheless, hormone levels were not measured with ultra-sensitive assays, which demonstrate a more reliable depiction of hormone levels in children. The mechanism by which drugs can cause gynecomastia is not always clear. However, various pathophysiologic mechanisms have been described. Some are directly related to increased serum estradiol levels or activation of estrogen or progesterone receptors in breast tissue, for example exogenous estradiol therapy. Other are related to blockage of dopamine D2 receptors, which may lead to hyperprolactinemia and can subsequently cause secondary hypogonadism by inhibiting LH and FSH. Other mechanisms include inhibition of CYP3A4, which is catalyzer of estradiol to 2- hydroxyestradiol [12, 13]. The exact mechanism by which ivacaftor can cause breast development is unknown. However, ivacaftor is a mild inhibitor of CYP3A4. Therefore, it is plausible that ivacaftor can increase the serum concentration of estradiol, especially when used concomitantly with other CYP 3A4 inhibitors [10]. In the presented clinical study all patients were treated with the same, recommended dose of ivacaftor [10]. As T_{max} of ivacaftor is 3–6 h, blood samples were taken 4 h after ingestion. In 5 patients additional blood samples were taken 3 and 5 h after ingestion which showed similar plasma concentrations as observed in the samples taken 4 h after ingestion. No dose-response relationship was found, which might suggest that the current recommended dose of ivacaftor is at the top of the dose-response curve. Absorption of ivacaftor is enhanced when

taken with fatty foods, which suggests that the absorption rate might be higher in pancreas sufficient patients. Most dose finding studies for ivacaftor are performed with pancreas insufficient patients and it is likely that plasma concentrations are higher when pancreas sufficient patients are treated with the same recommended dose. Moreover, studies have reported preservation or even restoration of pancreas function after treatment with ivacaftor [14, 15]. This might implicate that some patients, especially pancreas sufficient patients, can also be treated with a lower dose with the same effect. Moreover, concomitant use with other CYP 3A4 inhibitors could lead to higher plasma concentrations. As described by Guimbellot et al. [16] plasma concentration is a reliable indicator of the cellular concentration of ivacaftor. They describe a positive correlation between plasma concentrations and the in vivo cellular concentrations of ivacaftor. The cellular concentrations were considerably higher than the plasma concentrations, which suggests cellular accumulation of ivacaftor.

Conclusion

Findings from our case and patient cohort suggest that the currently advised dosages of ivacaftor might be at the top of the dose- response curve and in some patients can even be too high. Besides body weight, pancreas sufficiency and use of co-medication could possibly play a role in the plasma concentration of ivacaftor and occurrence of side effects. In patients with side effects of ivacaftor, a dose decrease should be considered while monitoring the clinical parameters. In patients without side effects studies with lower dosages are advocated to evaluate the added value of implementing personalized dosing regimens and to improve cost-efficacy of treatment.

Acknowledgments

We thank all patients who gave informed consent for participating in the clinical trials and all the members of the research teams that contributed to this work.

Supplemental table 1*: Co-medication during treatment with ivacaftor.*

Medication	N
beta-mimetics	6
inhaled steroids	8
nasal steroids	11
pulmozyme (dornase alfa)	11
inhaled antibiotics (tobramycine or colistin)	12
oral antibiotics	16
pancreatic enzymes	13
insulin	2
ursochol	1
vitamins, minerals	16

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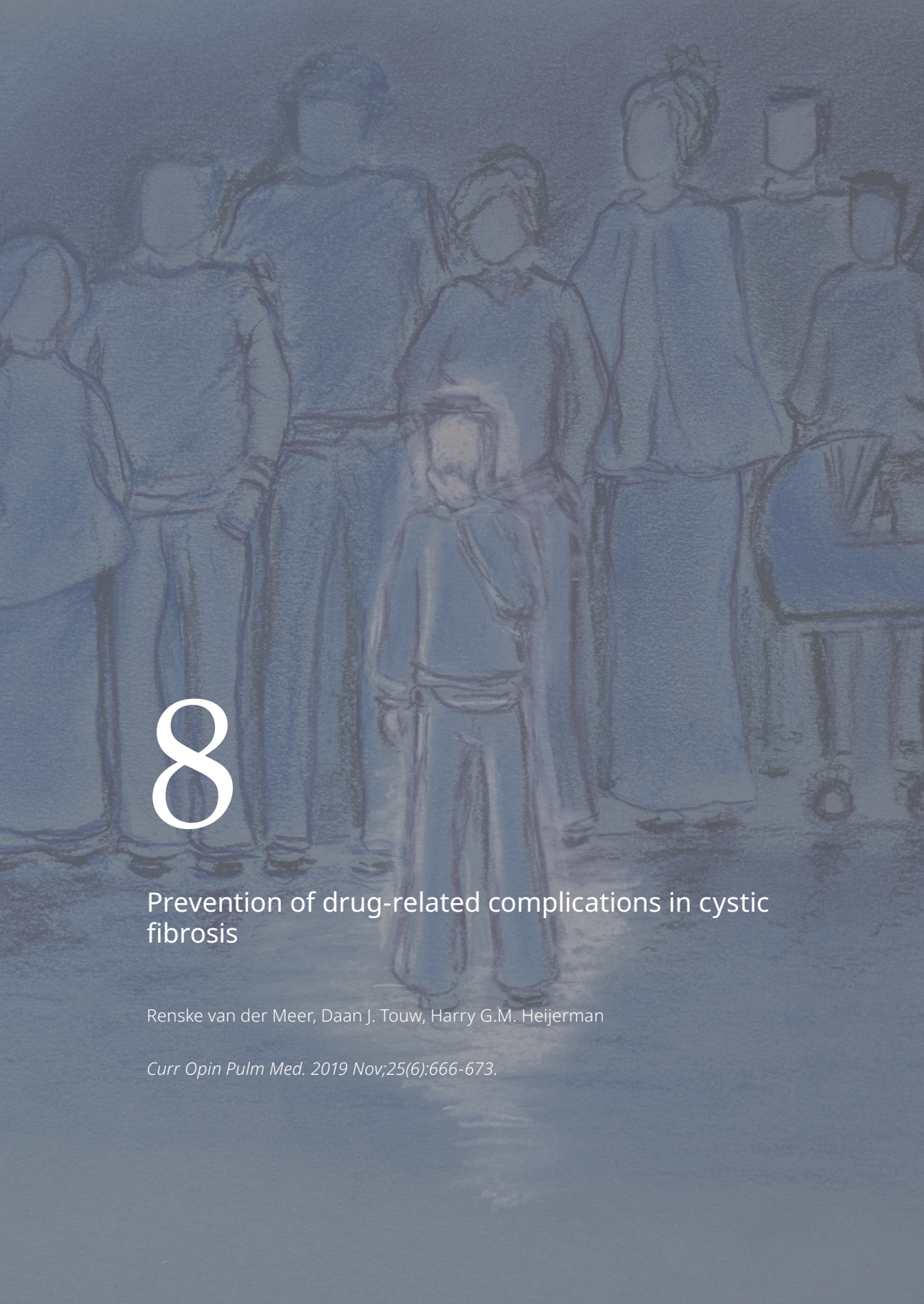

8

Prevention of drug-related complications in cystic fibrosis

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Purpose of this review

Due to continuous development of new drugs and better treatment strategies, survival of patients with cystic fibrosis has changed dramatically. Recently, targeted therapy of cystic fibrosis transmembrane conductance regulator (CFTR) modulators have become available. Despite these promising developments, treatment of this complex multiorgan disease constitutes a high and variable amount of other drugs. Complications of pharmacotherapeutic treatment are, therefore, expected to become more prevalent. This gives cause to review drug-related side effects in this new era in cystic fibrosis treatment.

Recent findings

We will discuss cystic fibrosis-related pharmacotherapies with a focus on indication of treatment, side effects and their complications, drug–drug interactions, and options to monitor and prevent drug-induced toxicity. Many recent publications about pharmacotherapy in cystic fibrosis, focus on antifungal therapy and CFTR modulators. We will give an overview of the most important studies.

Summary

With increased life expectancy which is, in part, because of better treatment options, the burden of pharmacotherapy in cystic fibrosis patients will increase. This has a high impact on quality of life as pharmacotherapy is time consuming and may cause side effects. Therefore, it is very important to be aware of possible pharmacotherapy-related side effects and their complications, drug–drug interactions, and options to monitor and prevent drug-induced toxicity.

Introduction

Cystic fibrosis is a multiorgan, autosomal-recessive disorder, and is the most common life-shortening hereditary disease in the Caucasian population. Cystic fibrosis is caused by mutations in the gene that encodes for a protein called the cystic fibrosis transmembrane conductance regulator (CFTR). This protein, an epithelial chloride channel, has important regulatory functions in various organs, including the pancreas, intestines, lungs, and liver. Severe lung disease is the most serious and progressive aspect of cystic fibrosis and is the main cause of morbidity and early death. Most therapies for cystic fibrosis treat the secondary consequence of the disease and focus either on reducing bacterial infection and inflammation or improving the nutritional and growth aspects of the disease. However, recently multiple compounds have been identified that target mutation-specific defects of the CFTR protein. This development of new therapies may lead to an increased life expectancy with a current median-predicted survival of approximately 50 years. Despite these recent developments, patients still are treated with a wide diversity of drugs often in high dosages. This has a high impact on quality of life as pharmacotherapy is time-consuming and may cause side effects. The relevance of drug-related complications has been clearly described by Peckham and Whitaker [1] in 2013. The recent development of an abundance of new drugs marks the beginning of a new era in cystic fibrosis treatment. This gives cause to review and revise cystic fibrosis therapies with a focus on indication of treatment, side effects and their complications, drug–drug interactions, and options to monitor and prevent drug-induced toxicity.

Pancreatic enzyme replacement therapy

Pancreatic enzyme replacement therapy (PERT) is standard care in cystic fibrosis patients with documented fat malabsorsorption. By combining PERT with a high-fat, high-energy diet, the nutritional status and thereby survival has improved [2]. No serious adverse effects are seen in patients treated with PERT. Most common side effects are stomach pain, nausea, and headache [3]. However, in 1994, Smyth et al. were the first to describe four cases of fibrosing colonopathy, a serious complication of PERT [4]. The pathogenesis is unknown, but may be associated with pancreatic enzyme dose [5], but constituents in the enteric coatings to protect the enzymes against gastric acid degradation may play a role as well [6]. The current recommendation to prevent fibrosing colonopathy is not to increase the enzyme dose without indication and to use less than 10.000 U lipase/kg/day [7, 8].

Antibiotics

Adverse reactions to antibiotics in patients with cystic fibrosis are a growing problem. During their lives, cystic fibrosis patients are exposed to a wide range of antibiotics. In order to reduce symptoms and prevent loss of pulmonary function, pathogenic microorganisms are treated with maintenance antibiotics administered via the inhaled, oral, or combined route. In case of an acute exacerbation, patients are mostly treated with (a combination of), intravenous antibiotics. Choosing the best antibiotic regimen for a patient can be a challenge, because of multiresistant pathogens and side effects of several antibiotics. A common problem when choosing an antibiotic regimen is the high amount of documented drug allergies in a patient. Often, these so-called allergies are adverse reactions of a different nature than immune-mediated hypersensitivity reactions (HSR). When observing an adverse reaction to medication, it is important to determine the type of reaction as being immune-mediated or not. If immune-mediated, the type of reaction should be specified. A hypersensitivity reaction type 1, occurs immediately after drug exposure, and includes symptoms like bronchoconstriction, urticaria, angioedema, or hypotension. A hypersensitivity reaction type 2, also known as a delayed type reaction, occurs days or weeks after exposure. These delayed type reactions might not only appear as maculopapular rash but also as severe reactions like SCAR (severe cutaneous adverse reactions), and DRESS (drug reaction with eosinophilia and systemic symptoms). The epidemiology and pathogenesis of hypersensitivity reactions are recently described by Wright et al. [9]. Petroni et al. [10] evaluated cystic fibrosis patients referred to an allergy clinic, because of an allergic hypersensitivity reaction (patients with a severe delayed type reaction with strict contraindication for the drug were excluded). Antibiotic testing, by skin prick, was performed at least 6 weeks after the suspected reaction. If this test was negative and there was no history of a severe adverse reaction, an antibiotic challenge with relevant antibiotics was recommended in order to classify drug reactions into three groups: able to use, desensitization needed, or strictly prohibited. In total, 17 challenges in 11 patients were performed, showing no hypersensitivity reactions or severe delayed reactions [10]. In case of exhausted treatment options because of adverse reactions on antibiotics, referral for an adequate drug allergy evaluation may be considered.

Nebulized antibiotics

Most adult cystic fibrosis patients are treated with inhaled antibiotics because of a chronic pulmonary infection with harmful microorganisms associated with worse clinical outcome [11]. There are several inhaled antibiotics available, but finding the optimal treatment regimen for one individual patient remains challenging.

Recently, levofloxacin became available for nebulization, showing a comparable efficacy to tobramycin inhalations [12]. However, levofloxacin has a bad taste and its use is contraindicated in patients with tendon complaints because of the use of fluoroquinolones. The advice to eat some chocolate or peppermints after inhalation is effective in some patients [12,13]. Several other nebulized therapies are in development including fosfomycin/tobramycine, ciprofloxacin dry powder inhalation, and liposomal amikacin. The latter being of great importance regarding the increase in number of nontuberculous mycobacterial infections [14]. Administering antibiotics by inhalation has the advantage of achieving high concentrations of a drug at the site of infection with limited systemic absorption. Nebulized antibiotics are often well tolerated without clinically important adverse events. Dry powder inhalations of tobramycin as well as colistin were reported to have the same safety profile as compared with nebulized tobramycin. Dry powder inhalers are easier and faster to use, which improves treatment adherence [15,16]. Most common side effects are because of local reactions. A bad taste in the mouth, cough, and chest tightness are most prevalent. Whenever starting a new inhalation treatment, one should actively look for bronchoconstriction by asking the patient if they experience any adverse effect and if so, perform a spirometry before and after inhalation of the antibiotic. In some patients, administration of a short-acting bronchodilator prior to the nebulized antibiotic may help to prevent bronchoconstriction [17]. In order to detect adverse events immediately, many centres perform an inhaled antibiotic trial in the hospital when initiating a new antibiotic. In very rare cases, systemic absorption of nebulized aminoglycosides may be significant enough to produce toxic effects, such as renal and vestibular toxicities [18]. In patients already showing signs of renal impairment or ototoxicity, inhalation with aminoglycosides should be used with great caution. In these patients, it may be useful to measure serum concentrations of the nebulized aminoglycoside in order to evaluate its potential harm.

Oral antibiotics

Macrolides

Cystic fibrosis patients may benefit from maintenance treatment with macrolides because of their antibacterial and presumed anti-inflammatory effect. Azithromycin reduces the number of pulmonary exacerbations and improves lung function in cystic fibrosis patients [19–21,22]. Azithromycin has a favourable safety profile, however, reports indicate rare cases of cardiac torsades des pointes in patients at risk. Macrolides can prolong the QT and QTc interval and cause cardiac arrhythmias, because of their effect on the potassium channel, although this is less likely to occur in cystic fibrosis patients [23]. Of all macrolides, azithromycin is least likely to cause

cardiac arrhythmias [24]. The vast majority of patients developing arrhythmias during treatment with macrolides have at least one additional risk factor [25]. Without additional risk factors for repolarization disorders, the incidence of arrhythmias in response to macrolides is estimated very low, less than one in 100.000 non cystic fibrosis individuals [26]. To decrease the incidence of macrolide-associated arrhythmias, it may be helpful to obtain patients' history regarding heart failure (including family history), and to review their concomitant medications. The latter may well clarify if they are on any medication that causes prolongation of QT interval. It is advisable to perform an ECG before the start of treatment and repeat an ECG while on treatment, particularly in case of an increased risk. The optimal duration of treatment with azithromycin is unclear. In most studies, patients were followed during 3–12 months to investigate the effect of treatment. Tramper Stranders et al. [27] and Willekens et al. [28] showed a positive effect on FEV1 and pulmonary exacerbations in the first year; however, the clinical benefit was not sustained in the second and third year of treatment. Currently, a topic of growing concern is the increase in macrolide-resistant strains of bacteria [29,30]. On the other hand, Cogen et al. [31] showed, in a predominantly paediatric cohort, a lower risk of several cystic fibrosis-related pathogens in patients chronically treated with azithromycin. Recent studies addressed the possible antagonizing effect of oral azithromycin in combination with inhaled tobramycin in cystic fibrosis patients colonized with pseudomonas aeruginosa [32]. Moreover, recent studies have shown the potential interaction between azithromycin and intravenous tobramycin in the treatment of pulmonary exacerbations may lead to a less favourable response to intravenous tobramycin [33,34]. The possible clinical impact must be further investigated.

Intravenous antibiotics

Pulmonary exacerbations are common in patients with cystic fibrosis and have considerable impact on patients' quality of life, pulmonary function and life expectancy [35,36]. In daily practice, a combination of intravenous antibiotics is often used to treat exacerbations. Synergistic effect and reducing the risk of antimicrobial resistance are potential factors favouring combination therapy [37–39]. On the other hand, more antibiotics are accompanied by a higher risk for toxicity and higher costs. In order to prevent toxicity of intravenous antibiotics, it is important to reduce the duration of treatment and to prescribe the optimal dose. Exacerbation management varies widely between cystic fibrosis centers, and the optimal treatment policy is not known. Recently, the 'STOP Standardized Treatment of pulmonary exacerbations) study team' investigated the management of pulmonary exacerbations(PEx) in cystic fibrosis with the final goal of defining best practices in the treatment of pulmonary exacerbations [40]. They showed a wide variation in antibiotic regimens and duration of treatment.

The mean (SD) duration of intravenous antibiotic treatment was 15.9 (6.0) days [41]. The 'STOP two trial' is now being implemented in order to investigate patients' response by means of lung function and symptom scores, measured after 1 week of antibiotic treatment. Patients are then divided in early responders and nonearly responders and randomized to an appropriate antibiotic duration. This study may provide better insight in the optimal treatment duration for our patients [42]. Pharmacokinetics of drugs eliminated by renal excretion are different in cystic fibrosis patients compared with the noncystic fibrosis population [43,44]. Therefore, therapeutic drug monitoring is standard care in intravenous treatment with aminoglycosides in order to achieve optimal antibiotic exposure and reduce toxicity [45]. The most serious side effects of aminoglycosides are nephrotoxicity and ototoxicity. Although nephrotoxicity is generally reversible by hydration and discontinuation of aminoglycosides, ototoxicity is often irreversible. In order to detect nephrotoxicity in an early stage, renal clearance and through concentrations (the lowest concentration of a drug before the next dose is administered) must bemonitored, at least weekly. Ototoxicity may be auditory or vestibular. The severity and type of toxicity depends on the type of aminoglycoside. Risk factors for ototoxicity are the total number of courses of intravenous aminoglycosides, mutations of the mitochondrial DNA, MTRNR1 gene, especially the m1555ANG mutation [46]. Patients are often asymptomatic making it essential to screen for early ototoxicity by performing audiometry. Extended high-frequency (EHF) audiometry identifies more children with ototoxicity than standard pure tone audiometry (PTA) [46].

Antifungals

Aspergillus fumigatus is frequently cultured in cystic fibrosis patients [47]. About 10% of the colonized patients develop aspergillus sensitization or allergic bronchopulmonary aspergillosis (ABPA) [48]. Rarer clinical presentations of aspergillus disease are aspergillus bronchitis [49], aspergilloma, and invasive pulmonary aspergillosis [50]. The treatment of ABPA consists primarily of steroids. In case, a patient does not respond to steroids or cannot tolerate them, antifungal therapy may be considered to reduce the burden of aspergillus fumigatus allergens. However, studies show conflicting results [51,52]. A complicating factor of combination treatment of (inhaled) steroids and azoles is the increased risk of side effects including Cushing syndrome, osteoporosis, and cataract [53]. Data about omalizumab for treatment of ABPA in cystic fibrosis patients wherever steroids failed are scarce [54], and randomized trials are lacking. To date, there is no firm recommendation when and how to use omalizumab for ABPA in patients with cystic fibrosis.

Triazoles are the most commonly used antifungals in the treatment of aspergillus disease. Currently fluconazole, itraconazole, voriconazole, posaconazole, and isavuconazole are available. Common adverse events of all the triazoles are abdominal pain, nausea, vomiting, and diarrhoea. In a noncystic fibrosis patient group hepatotoxicity occurred in 25% of the patients treated with azoles. Therefore, it is important to check liver chemistry regularly. Peripheral neuropathy is an underestimated problem related to chronic use of azoles that one should be aware of [55]. Performing an ECG and checking comedication is important because of the possibility of a prolonged QT interval and the large amount of drug–drug interactions because of their influence on cytochrome P450 [56]. Azoles in cystic fibrosis patients have higher inter-patient as well as intra-patient pharmacokinetic variability than in healthy volunteers [57]. Therefore, therapeutic drug monitoring (TDM) is needed to optimize the effect and safety and to prevent azole resistance [58]. TDM of itraconazole, voriconazole and posaconazole indeed improves patient's outcome and minimizes toxicity [59,60].

Azole-resistant aspergillus fumigatus (ARAF) is an increasing problem, especially in cystic fibrosis patients, with a prevalence of around 8% [61,62]. In order to prevent unnecessary toxicity of azoles, susceptibility testing before starting treatment should be considered. Whether combination therapy with two or three antifungals leads to a better outcome than treatment with a single azole in patients with ARAF is not known. In scedosporium-infected cystic fibrosis patients, data from a recent observational study favour the use of combination therapy [63]. The effect of treatment with formulations of amphotericin B or an echinocandin in aspergillus disease in the cystic fibrosis population is not known and needs to be investigated in future studies. In immunocompromised patients with refractory invasive aspergillosis, antifungal combination therapy is often employed in order to improve outcomes of a single antifungal drug [64]. Most in-vitro studies demonstrate synergistic or indifferent interactions [65,66] but in-vivo studies show no significant improvement compared with monotherapy [67].

Cystic fibrosis transmembrane conductance regulator modulators

CFTR modulators are a new class of medication targeting the underlying defect in cystic fibrosis. With the development of the CFTR modulators, a new era in cystic fibrosis treatment has arrived. To date, CFTR modulators include potentiators and correctors.

Potentiatiors

Potentiators have the capacity to increase channel opening of CFTR at the cell surface [68]. Ivacaftor, currently the only CFTR potentiator approved for clinical use, has demonstrated a clinically relevant effect in people with class III and class IV mutations (R117H) [69,70]. Ivacaftor has a favourable safety profile and has not been associated with significant pulmonary symptoms. Like all modulators, ivacaftor may cause hepatotoxicity. Transaminitis and some cases of more severe hepatic dysfunction were seen in ivacaftor studies, therefore, monitoring of hepatic function is recommended. Childrenless than 12 years of age require serial opthalmologic evaluation because of the possible development of cataract [71–73]. Drug–drug interactions with CYP3A inhibitors (such as azoles and macrolides, not azithromcyin) require dose reduction of ivacaftor while combining these therapies. On the other hand, rifamycins will reduce the ivacaftor concentration, which makes this therapy useless. Dose adjustment and therapeutic drug monitoring may, therefore, be helpful. However, currently there is no commercially available clinical test for TDM of modulators. A new potentiator, deuterated ivacaftor, has a better pharmacokinetic and similar safety profile compared with ivacaftor [74]. Currently, phase two clinical trials with deuterated ivacaftor are being performed.

Correctors

Correctors are CFTR modulators that are able to partially correct the folding defect in F508del-CFTR, which results in an increased amount of surface protein [68]. Lumacaftor and tezacaftor are first generation CFTR correctors. In combination with ivacaftor, lumacaftor has clinical benefit in patients with cystic fibrosis homozygous for F508del-CFTR [75–77].

Recent trials in F508del patients have demonstrated a generally comparable improvement in lung function on tezacaftor/ivacaftor therapy as compared with data fromlumacaftor–ivacaftor trials [78]. Tezacaftor/ivacaftor was also efficacious in studies with patients with F508del/residual-function mutations, and slightly better in patients with F508del/G551D CFTR than ivacaftor monotherapy [79,80].

Lumacaftor is a strong CYP3A inducer having the highest number of drug–drug interactions of all current modulators. In the patient package insert is mentioned that with simultaneous use of lumacaftor/ivacaftor and hormonal contraceptives, these birth control methods may become ineffective (Incorporated VP, editor. Package Insert Label: Product Monograph Orkambi). One may advise female patients to use an intrauterine device (IUD, Mirenaor copper) as a well-tolerated contraceptive therapy [81]. Serum concentrations of other drugs such as selective serotonin reuptake

inhibitors and azoles are reduced and may decrease below therapeutic range. On the other hand, azoles inhibit the elimination of CFTR potentiators [82]. Therapeutic drug monitoring measuring serum concentrations of azoles is, therefore, strongly recommended. If, despite dose adjustment, serum levels remain sub-therapeutic, one may consider the pros and cons of decreasing the dosage of lumacaftor/ivacaftor. However, recommendations in the summary of product characteristics are lacking. Lumacaftor also gives a possible decrease in serum levels of corticosteroids, which might complicate the treatment of allergic bronchopulmonary aspergillosis.

The first real life experience with lumacaftor/ivacaftor was in a compassionate use program for patients with a rapid decline in lung function and/or a FEV_1 below 40% predicted. In these patients, respiratory adverse events were more common than in patients with a FEV_1 above 40% predicted [83]. Side effects often cause patients to stop treatment.

Two advantages may favour the use of tezacaftor/ivacaftor above lumacaftor/ivacaftor: including a better tolerability and less drug–drug interactions. In the EXPAND [79] and EVOLVE trials [78], no patients discontinued the study because of chest tightness, whereas this was a major problem when starting on lumacaftor/ivacaftor. Regarding drug–drug interactions, tezacaftor/ivacaftor allows women to reliably use hormonal contraception, in contrast to lumacaftor/ivacaftor. However, as with lumacaftor/ivacaftor, use of strong CYP3A inducers is not recommended and the dose of tezacaftor/ivacaftor should be reduced when co-administered with tezacaftor/ivacaftor. Consider that therapeutic drug monitoring of the azoles is a useful course of action.

Fortunately, the development of next-generation CFTR correctors looks very promising. Phase two trials of next-generation CFTR correctors (VX 445 and VX 659) in combination with tezacaftor/ivacaftor showed acceptable safety profiles and an increase of percentage of FEV_1 predicted up to 13.8 points in patients homozygous for F508del and heterozygous for F508del/minimal function [84,85].

Conclusion

Continuous improvement in the treatment of cystic fibrosis patients has led to a significant increase in life expectancy from approximately 20 years in the sixties to 50 years for patients born in the early 2000s. With the development of CFTR modulators, a promising era of targeted therapy has commenced. Current developments might further increase life expectancy of many patients. However, multiple drugs will still be needed to treat this complicated disease. Together with prolonged survival, complications of treatment and side effects will become more common, making it important to monitor these drug-related problems. Before starting a new drug, one should carefully consider the pros and cons of a certain therapy, including its possible harm and the potential development of antimicrobial resistance. Even though these are common issues in daily care, many questions have not been fully answered yet, including issues regarding the optimal duration of therapy, the optimal drug dose or the use of combination therapy. Even the need to treat might be under debate. In order to reduce treatment burden and the risk of complications, it is essential to find answers to these many questions by performing well designed clinical trials.

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9

General discussion

In this thesis we show several aspects of the clinical pharmacology of cystic fibrosis transmembrane receptor (CFTR) modulator treatment. By investigating pharmacokinetic (PK) and -dynamic(PD) features of these drugs in groups of people with CF (pwCF) with different phenotypes of the disease, we aimed to improve the insight into the possible causes of the diversity in treatment effect as has been observed in clinical practice.

In this chapter we will discuss characteristics of pwCF that can contribute to differences in treatment response: what is known and what needs to be elucidated?

We will reflect on the pitfalls of our studies and finally we will describe future strategies needed to get insight in which individuals may benefit from dose adjustment and the role of TDM in dose optimization.

Influence of CFTR mutation on treatment effect of CFTR modulators

More than 1700 mutations lead to cystic fibrosis, causing various defects in CFTR expression, folding, and channel function [1]. CFTR modulators have been developed to revert the effects of the disease-causing mutations [2]. Extensive research has been done to uncover the mechanisms of CFTR modulators. Whereas the structural and functional basis of action of the potentiator ivacaftor has been described [3], the mechanism of correctors remains largely undefined. CFTR correctors have been categorized into different clusters based on their functional redundancy or additivity. Correctors from different clusters act through different mechanisms, and some can be combined to synergistically improve CFTR folding [4].

Predicting the clinical response of a certain CFTR modulator (combination) in pwCF and a certain CFTR mutation is difficult, especially for rare mutations. Rectal organoids, *in vitro* primary cell cultures, are developed and help in predicting drug response [5, 6]. The value of the use of organoid models in predicting treatment response is highlighted in chapter 6. The hypothesis of this study was based on *in vitro* data showing swelling of F508del/A455E organoids after incubation with lumacaftor/ivacaftor [6]. Although the sample size was small and the study duration relatively short, the results suggest a clinical benefit from lumacaftor/ivacaftor in pwCF and a A455E mutation.

It is important to realize that clinical benefit depends on the outcome measure and the baseline characteristics (irreversible or reversible damage). Therefore, pulmonary function might not be the best parameter to look at for all pwCF. CFQ-R, including the non-respiratory domains of the CF questionnaire may give good insight in the

symptoms of this multi organ disease in an individual with CF. Although the change in sweat chloride concentration has the advantage to be independent of clinical manifestations of CF disease, its repeatability is moderate.

Patient characteristics that may alter pharmacokinetic properties of CFTR modulators

Pharmacokinetics show what the body does to the drug. Different features of CF disease may influence pharmacokinetic properties of drugs which may contribute to variation in drug exposure. In paragraph 4 of chapter 2 features of CF disease are described that may change pharmacokinetic properties by changing the absorption, distribution, metabolism or elimination of CFTR modulating drugs.

As CFTR modulators are substrates of cytrochrome P450 3A4 (CYP3A4) and cytochrome P450 3A5 (CYP3A5), drug-drug interactions (DDI's) with inhibitors or inducers of these enzymes may occur and thereby change drug exposure [7-10]. These DDI studies were performed in healthy volunteers and not in CF patients. In chapter 3 we show results of a DDI study in both healthy subjects and subjects with CF. The interaction potential of CYP3A4 inhibitors (azithromycin, clarithromycin and ritonavir) on ivacaftor was the same in subjects with CF as in healthy volunteers. The exposure to ivacaftor was increased 7-8 times by co-administration of ritonavir and 3 times by co-administration of clarithromycin. This DDI could be used to lower the dose of CFTR modulators and thereby save costs. A practical and safe option may be to replace azithromycin, used chronically by many pwCF, by clarithromycin. An interesting result of this study was the almost two times lower exposure (expressed in AUC) to ivacaftor in subjects with CF, with a higher T_{max} and a lower C_{max} and $T_{1/2}$ suggesting a more gradual uptake of ivacaftor in CF patients and a reduced bio-availability. All participating subjects with CF were pancreas insufficient (PI). The profound influence of fat containing food compared to fasting and the reduced level of exposure in PI pwCF compared to healthy volunteers gave rise to investigate the influence of exocrine pancreatic function and pancreatic enzymes on the absorption and exposure to ivacaftor. We performed a study (presented in chapter 4) in 10 PI subjects with CF and 10 pancreatic sufficient (PS) pwCF. Since PI pwCF still suffer from fat malabsorption despite treatment with pancreatic enzymes [11], we also investigated the influence of pancreatic enzymes on ivacaftor absorption in the PI participants. The results of this study show no significant difference in the rate of absorption nor exposure of ivacaftor between PI and PS subjects. Also pancreatic enzymes did not change these pharmacokinetic parameters in PI subjects. CFTR modulators are hydrophobic and lipophilic compounds and intake together with fat is expected to increase dissolution of ivacaftor and thereby enable the absorption. Therefore, the advice to administer these drugs with fat containing

food is maintained regardless of the exocrine pancreatic function. In clinical practice, the majority of PI pwCF will take their pancreatic enzymes with their food to prevent abdominal complaints.

Although for many DDI's with CFTR modulating drugs a dosing advice has been described in the SmPC documents [7-10], for some drugs as tacrolimus, the expected DDI gives reason to be reluctant starting CFTR modulator treatment. With the availability of highly effective CFTR modulators the need to examine this DDI and the safety of co-administration of these drugs became urgent, especially for patients with a history of solid organ transplantation other than lung transplantation. In chapter 5 we present a DDI study of elexacaftor/tezacaftor/ivacaftor (ETI) in pwCF using tacrolimus after a kidney or liver transplantation. We showed that despite the narrow therapeutic window of tacrolimus, no side effects related to DDI occurred. Dose adjustments were needed in most patients at 10-14 days after starting ETI. However, the DDI could be managed by close monitoring of tacrolimus trough levels Studies with a long-term follow up are needed to gain better insight into the long-term effects and safety on the longer term (e.g. hepatotoxicity, allograft function). Recently, a physiologically based pharmacokinetic (PBPK) model has been developed to predict DDI between ETI and tacrolimus in pwCF after lung transplantation. Such models may be useful in predicting the appropriate dosing regimen to manage the risk of DDI [12].

With the better and longer life expectancy of many pwCF using ETI, the landscape of treatment of this multi organ disease is expected to change. In the aging CF population, other comorbidities will emerge, also those that are unrelated to CF disease. Therefore polypharmacy will still be a problem, especially in older pwCF, with a high risk of side effects and DDI's. In chapter 8 we therefore reviewed drug induced side effects in pwCF with a focus on how to manage these complications. However, with the new landscape of CF phenotype and treatment, also complications and DDI's are expected to change.

Why is understanding the exposure-clinical response relationship of CFTR modulators important?

CFTR modulators are prescribed at standardized dosages. Several circumstances such as clinical toxicity, less-than-expected clinical response, drug or food interactions, distinct patient subgroups (i.e. patients after organ transplantation, patients with liver disease), may give a need to adjust the dosage. An illustrative example of the importance of understanding dose-response relationships is a case described in chapter 7. Due to side effects (breast development) the dosage ivacaftor has been decreased with complete recovery of side effects and a stable clinical condition (Forced

expiratory volume in 1 second (FEV_1) and sweatchloride). Currently more reports are published describing cases with side effects of CFTR modulating therapy with a need for decreasing the dosage. In most of the cases side effects disappear after dose reduction and clinical parameters remain stable [13]. This illustrates that patients may benefit from personalized dosing regimens.

Therapeutic drug monitoring: future perspectives.

An important result of the pharmacokinetic studies presented in this thesis (chapter 3, 4 and 5) is the high between-patient-variability in drug exposure of the examined CFTR modulators (ivacaftor and ETI). However, the impact on treatment tolerability and clinical response is yet unclear. This gives rise to the question if TDM of CFTR modulators will be a useful tool to determine the optimal dosing regimen.

In daily practice, blood concentration measurement of drugs is an important tool to optimize the use of critical drugs by adjusting drug exposure via a therapeutic drug monitoring (TDM) program [14, 15]. Although plasma concentrations are a frequently used indicator to predict therapeutic efficacy, for CFTR modulators, the lung tissue concentrations would ideally be obtained to correlate with the treatment effect on the CFTR protein located within the lung. However, lung tissue concentrations are not easily accessible, in contrast to blood or plasma that is minimally invasive for patients. The question is if CFTR modulators are suitable candidates for therapeutic drug monitoring. In figure 1, three criteria for drugs needed to be candidate for TDM are shown.

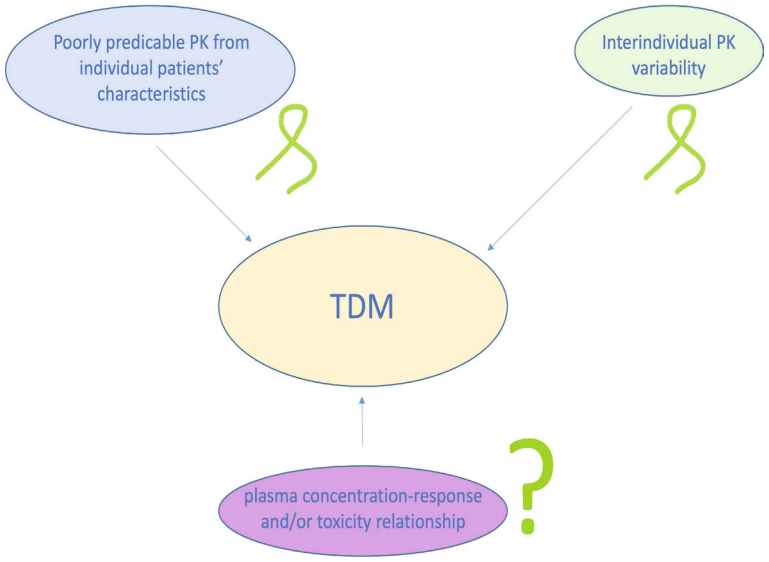

Figure 1. *Criteria for drugs to be candidates for TDM.*

PK=pharmacokinetic(s).

1. *Is the PK variability of CFTR drugs significant?* ***Yes.***
Patients receiving ETI (but also other CFTR modulator (combinations)) show high standard deviation values for PK parameters [16]. This variability is also illustrated by the results of the PK studies of this thesis. The $AUC_{0\text{-}12}$ measured in steady state for ivacaftor in PS pwCF using tezacaftor/ivacaftor was 15,42 (6.93-48,05 ug.hr.ml_1) median (min-max) as shown in chapter 4. The trough levels of ivacaftor,tezacaftor and elexacaftor in subject using tacrolimus were (median (min-max)) 0,58 (0,38-1,23)µg/mL, 1,63 (0,90-2,99)µg/mL and 4,87 (3,64-10,26)µg/mL respectively (chapter 5).

2*. Is the PK variability poorly predictable from patients' characteristics?* ***Yes.***
Information on plasma concentrations of CFTR modulators is scarce. The exposure to ivacaftor in subjects with CF in our study was almost two times lower as compared to healthy volunteers (chapter 3). Also Hanafin et al. observed a lumacaftor exposure in CF patients of almost half of that measured in healthy controls [17]. The reason for this difference is unclear. We suggested this was caused by fat malabsorption which was present in all of the participating pwCF in our study. However, this hypothesis could not be confirmed by the results of our study comparing pancreas insufficient with pancreas sufficient pwCF using tezacaftor/ivacaftor (chapter 4). Hanafin at al.

conducted a study with the objective to assess the impact of patient characteristics on the PK of ivacaftor/lumacaftor administered to pwCF at five different treatment sites. They observed that patient weight and age had a significant effect on the C_{max} of lumacaftor and ivacaftor-M1. Also they found differences in C_{max} values for ivacaftor and lumacaftor in participating centers in different countries, suggesting a relation between the type of food and PK. The study populations in the PK studies in this thesis were too small to underline these correlations. Although genomic profiling including cytochrome profile is not yet considered standard of care it might also affect drug exposure. Currently, dose adjustments are advised in case of impaired liver function, body weight below 25 kg and co-administration with certain drugs. If dose adjustment is needed for other reasons needs to be elucidated.

3. *Is the relationship between plasma concentration and clinical response and/or toxicity clear?* ***No.***
The "caftor" dose–response relationships with commonly used CF clinical outcome measures (body mass index (BMI), FEV_1, nasal potential difference (NPD), and sweat chloride concentration) have mainly been studied in dose-escalation regimens carried out in phase II studies with adult CF patients. A trend of increased response with higher doses was reported for ivacaftor, lumacaftor and tezacaftor monotherapy. No distinct dose–response was observed for elexacaftor for the studied 50–200 mg dosage range (see also chapter 2, paragraph 3.2 of this thesis). The high variability in treatment response found in patients with the same CFTR genotype and dosage regimen [18,19] suggests that interindividual differences in pharmacokinetics will, at least in part, be responsible for the inconstance in drug response.

Currently ETI is the most effective modulator combination and registered for pwCF with at least one F508del mutation (90% of the Dutch CF population). From clinical perspective, insight in the dose-exposure-clinical response relationship of ETI is of great importance.

Since the registration and reimbursement of ETI in many "high-income" countries more side effects of ETI are reported than expected based on registration studies. Many patients and CF physicians struggle with the management of these side effects. Sometimes this drug related toxicity leads to the decision to lower the dose with often resolvement of symptoms and clinical stability (measured by FEV_1 and sweat chloride) [13]. We are currently investigating data of 18 patients in our hospital in whom a dose reduction was performed because of side effects. In these patients we measured serum levels of ETI and clinical outcome after dose adjustment (FEV1, sweat chloride, side effects) at full dosage and after dose reduction.

Possible reasons for requesting drug concentrations measurement are:

- Suspected toxicity – toxic concentrations?
- Lack of response – subtherapeutic concentrations?
- Assessment of compliance with medication regimen
- Asses therapy after a change in dosage regimen
- Change in clinical state of the patient
- Potential DDI

All these indications may apply to pwCF on CFTR modulator treatment.

A major limitation of CFTR modulators, is the excessive costs when they reach the market. This renders difficulties in their availability for many pwCF [20, 21], especially for those living in low- and middle-income countries [22]. In the perspective of reducing costs and appropriate use of drugs, TDM might be helpful to adjust the most effective drug dosage for each individual.

Methods to improve our knowledge

As explained in the previous paragraph, TDM of CFTR modulators might be a useful tool to determine the optimal dosing regimen for each patient. However the knowledge of the relationship between plasma concentrations of CFTR modulators and their clinical response and/or toxicity is currently insufficient. In clinical practice, ETI is the most commonly used CFTR modulator combination. Determination of ETI blood levels in a large group of patients treated with the standard ETI dosage is a first step to provide insight into the variation of ETI levels in pwCF and the correlation between these bloodlevels and clinical outcome and/or toxicity. It may also reveal correlations between patient characteristics and ETI levels.

As mentioned in chapter 2, paragraph 3.3 several methods may be used in future studies to improve our understanding of the exposure-clinical response relationship of CFTR modulators and thereby determine the therapeutic window of these drugs.

- Plasma drug concentrations: Peripheral blood is easily accessible. Analytical methods have been developed and validated for rapid detection and quantification of ivacaftor, its major metabolites, lumacaftor, tezacaftor and elexacaftor in the plasma and sputum of pwCF [23, 24]. The plasma concentration of ivacaftor was correlated with cellular concentrations but the cellular concentrations were disproportionally more elevated in patients with higher plasma concentrations suggestion accumulation of ivacaftor in vivo [25].

- Cellular drug concentrations: CFTR modulators act within cells. Therefore ideally intra cellular drug concentrations (e.g.lung tissue concentrations) would be used to be related to treatment effect. However, lung tissue is not easily accessible.
- Physiologically based pharmacokinetic models (PBPK) to predict tissue concentrations are recently developed [26]. Hong et al. report in a clinical case series their experience of dose reduction in individuals who experienced adverse events following ETI therapy. They provide mechanistic support for ETI dose reduction by exploring predicted lung exposures and underlying pharmacokinetics-pharmacodynamics (PK-PD) relationships by the use of PBPK [22].
- Organoids. Dekkers et al. described a bioassay to measure CFTR modulator activity in human plasma using intestinal organoids. They observed a dose-dependent increase of forskolin-induced organoid swelling for ivacaftor [27]. Although this technique may help us to predict treatment response in vivo for patients treated with ivacaftor monotherapy. Its value for dose optimization, especially for ETI, is limited due to the very high response in organoid swellling.

Concluding remarks

Recent developments in CFTR directed therapeutics sheds light on barriers that must be overcome to allow efficient therapy for all individuals with CF. The prescription of CFTR modulators needs to be optimized with regard to clinical efficacy but also tolerability, long-term safety, and potential DDIs. In this thesis we tried to clarify the heterogenicity in clinical response to CFTR modulator treatment in pwCF with the same CFTR mutation. We showed that the fatmalabsorption due to pancreatic insufficiency did not change the absorption or exposure to ivacaftor. If other patient characteristics correlate with drug exposure needs further investigation with a focus on ETI. We also showed examples of DDI between CFTR modulators and other drugs that are important in CF treatment and how to manage these DDI in clinical practice. This opens doors for treatment with these highly effective modulators for more pwCF. In our studies, the observed variability of CFTR modulator exposure in pwCF using the same dosage was extremely high. Future studies are needed to evaluate the usefulness of TDM of CFTR modulators to determine the optimal dosing regimen for each patient.

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Summary in dutch

Nederlandse samenvatting

Hoofdstuk 1:
Inleiding

Cystic fibrosis (CF), ook bekend als taaislijmziekte is een erfelijke aandoening en wordt veroorzaakt door mutaties in het cystic fibrosis transmebraan receptor (CFTR) gen. Hierdoor ontstaat er een probleem met het CFTR-eiwit. Dit eiwit is belangrijk voor de zouthuishouding in de slijmvormende cellen en zweetklieren in het lichaam. Als je van beide ouders een verkeerd gen erft leidt dit tot CF. 1 op de 30 personen heeft een verkeerd gen, dit noem je drager. Een drager is niet ziek. In Nederland zijn er ongeveer 1500 mensen met CF. Als het CFTR eiwit niet goed werkt kunnen er problemen in verschillende organen ontstaan waaronder de longen, alvleesklier, lever, darm en de voortplantingsorganen. Er zijn veel verschillende soorten mutaties bekend in het CFTR gen. De ernst en uiting van de ziekte wordt mede bepaald door het soort mutatie. Het ziektebeeld CF is dus niet bij iedereen hetzelfde.

Sinds de ontdekking van het CFTR gen in 1989 is er veel veranderd in de behandeling van CF. Een belangrijke ontwikkeling enkele jaren geleden zijn de CFTR modulatoren. Deze medicijnen zijn de eerste medicijnen voor CF die de oorzaak van de ziekte aanpakken door de functie van het CFTR-eiwit te verbeteren in plaats van klachten te bestrijden. Momenteel heeft ongeveer 90% van de Nederlands CF patiënten een mutatie die geschikt is voor behandeling met CFTR modulatoren. Van hen krijgt (nog) niet iedereen deze behandeling bijvoorbeeld doordat ze te jong zijn of een orgaantransplantatie hebben ondergaan. Wereldwijd zijn er nog heel veel landen waar deze zeer dure medicijnen niet worden vergoed.

Het meest effectieve medicijn dat sinds 2022 vergoed wordt in Nederland bestaat uit een combinatie van 3 modulatoren: elexacaftor/tezacaftor/ivacaftor. Het effect van deze medicijnen wordt vooral beoordeeld door te kijken naar longfunctie (de hoeveelheid lucht die per seconde uitgeademd wordt: FEV_1) en het effect op de hoeveelheid zout in het zweet (hoe lager hoe beter). In onderzoeken zien we een duidelijk effect op groepsniveau (dus gemiddeld in alle patiënten) maar op individueel niveau zijn er ook patiënten met een heel beperkt effect of zelfs achteruitgang. Ook zijn er patiënten die veel bijwerkingen hebben van deze medicijnen en hierdoor soms zelfs stoppen met deze behandeling met vaak acute verergering van hun klachten als gevolg. De vraag is waarom de uitwerking van deze medicijnen zo verschilt tussen patiënten met dezelfde mutatie.

Het effect van een medicijn wordt bepaald door farmacodynamiek (wat doet het geneesmiddel met het lichaam) en de farmacokinetiek (wat doet het lichaam met het geneesmiddel). Overkoepelend noemen we dit farmacologie. In dit proefschrift ligt de nadruk op de farmacokinetiek waarin we verschillende factoren onderzoeken die de farmacokinetiek van CFTR modulatoren kunnen veranderen en daarmee ook de hoeveelheid medicijn waaraan de patiënt wordt blootgesteld. Hiermee willen we meer inzicht krijgen in de beste behandeling voor ieder individu met CF: een maximaal effect met minimale bijwerkingen.

Hoofdstuk 2:
Is voor iedereen dezelfde hoeveelheid van de CFTR modulator nodig voor het beste effect? Een kijkje in de farmacologie.

Zoals beschreven in hoofdstuk 1 is het gunstige effect van CFTR modulatoren op groepsniveau evident maar zijn individuele effecten van CFTR modulatoren variabel. Dit effect is een uiting van farmacodynamiek en farmacokinetiek.

Farmacodynamiek: CFTR modulatoren verbeteren de functie van het CFTR ion kanaal ofwel chloride kanaal. Deze medicijnen worden onderverdeeld in 2 groepen op basis van hun werking: potentiatoren en correctoren. Potentiatoren verbeteren het openen van het ion kanaal waardoor chloride transport toeneemt. Ivacaftor is op dit moment de enige geregistreerde potentiator. Correctoren verbeteren de vouwing en transport van het CFTR eiwit waardoor het minder wordt afgebroken en beter kan werken. Momenteel zijn er 3 geregistreerde correctoren: lumacaftor, tezacaftor en elexacaftor. Welk medicijn (of combinatie) het beste werkt hangt af van de CFTR mutatie.

Farmacokinetiek: De vraag is of dezelfde dosis van een medicijn (hier CFTR modulator) resulteert in een verschillende blootstelling (hoeveelheid in de patiënt) en daardoor een verschil in effect veroorzaakt wordt (fig 1). Hier heeft de ontwikkelaar van de modulatoren uiteraard onderzoek naar gedaan. Uit onderzoek valt echter op dat de hoeveelheid medicijn in het bloed erg verschilt tussen patiënten. Daarnaast zien we in de praktijk bij de geadviseerde dosering toch mensen met bijwerkingen en mensen zonder effect.

We weten dat niet iedereen met CF hetzelfde is en iemand met CF niet hetzelfde is als iemand zonder CF. Diverse kenmerken van een individu kunnen de farmacokinetiek beïnvloeden. We kijken hierbij naar 4 principes, de zgn “ADME” (fig 2): absorptie (opname van het geneesmiddel), distributie (verdeling over het lichaam), metabolisme (omzettting in (on)werkzame stoffen) en eliminatie (uitscheiding van het

geneesmiddel): deze 4 factoren leiden tot een bepaalde blootstelling in de patiënt (fig 1). Verschillende uitingen van CF, bijvoorbeeld een slecht werkende alvleesklier of een laag lichaamsgewicht kunnen (een van) deze principes veranderen. Dit wordt in hoofdstuk 2 uitvoerig beschreven. Hierdoor krijgt de lezer meer inzicht in de farmacologie van de CFTR modulatoren. Ook worden er ideeën besproken voor verder onderzoek.

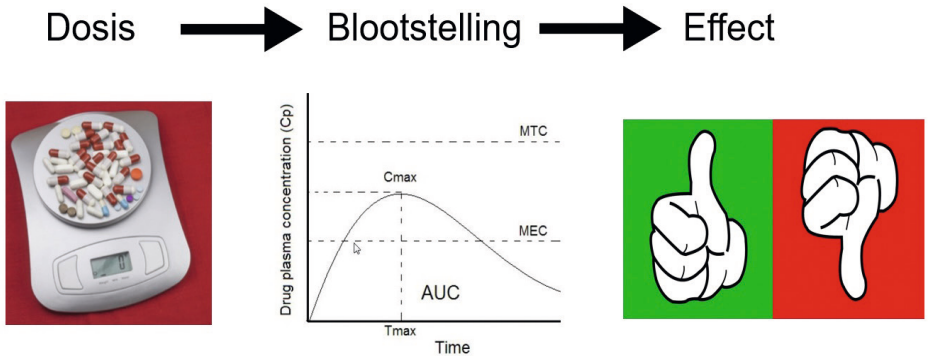

Figure 1. *Dosis-effect relatie*

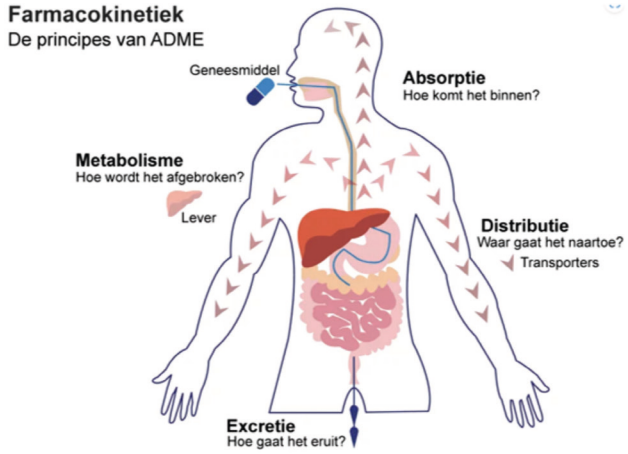

Figure 2. *"ADME" principes.*

Hoofdstuk 3:
Welke invloed hebben medicijnen die CYP3A4 enzymen remmen op de farmacokinetiek van ivacaftor: is dit gelijk voor gezonde mensen en mensen met CF?

Ivacaftor is de enige geregistreerde potentiator en wordt alleen of in combinatie met een of meerdere correctoren gebruikt. Ivacaftor wordt in de lever omgezet met name door het enzym CYP3A4. Veel mensen met CF gebruiken medicijnen die CYP3A4 remmen waardoor er een hogere blootstelling aan ivacaftor ontstaat. Het advies is dan ook om in zo'n situatie de dosering ivacaftor aan te passen. De huidige adviezen zijn gebaseerd op basis van onderzoek in gezonde vrijwilligers. We hebben daarom het effect van een sterke (ritonavir), matige (claritromycine) en milde (azitromycine) CYP3A4 remmer op de farmacokinetiek van ivacaftor onderzocht in gezonde mensen en in mensen met CF. We vonden dat de blootstelling aan ivacaftor in gezonde mensen hoger was dan in mensen met CF, dit verschil was opvallend maar niet significant. De sterke CYP3A4 remmer verhoogde de blootstelling in alle deelnemers met een factor 7 en de matige CYP3A4 remmer met een factor 3. Met deze uitkomst is er geen reden om de huidige adviezen over de dosis aanpassing bij gelijktijdig gebruik van ivacaftor en een CYP3A4 remmer te veranderen. Waarom de blootstelling aan ivacaftor hoger was in gezonde mensen moet verder onderzocht worden. Een van de verklaringen zou kunnen zijn dat de alvleesklier van de CF patiënten in dit onderzoek minder werkt. Dit wordt toegelicht in hoofdstuk 4.

Hoofdstuk 4:
Is de farmacokinetiek van ivacaftor veranderd in mensen met CF en een slecht werkende alvleesklier?

Alle CFTR modulatoren (behalve tezacaftor) moeten ingenomen worden met vet bevattend voedsel omdat dit de blootstelling aan het medicijn 4 keer verhoogd. Aangezien de alvleesklier een belangrijke rol speelt bij de vetvertering en deze functie bij veel mensen met CF gestoord is hebben wij onderzocht of in patiënten met zo'n slecht werkende alvleesklier (we noemen dit exocriene pancreas insufficientie) de opname van en blootstelling aan ivacaftor anders is dan in mensen met CF en een goed werkende alvleesklier. Hiervoor hebben we in totaal in 20 mensen met CF de ivacaftor opname en blootstelling onderzocht. 10 van hen hadden een goed werkende en 10 een slecht werkende alvleesklier. Zij gebruikten allemaal de CFTR modualoren tezacaftor/ivacaftor. Omdat mensen met een slecht werkende alvleesklier behandeld worden met alvleesklierenzymen hebben we ook het effect van wel of niet innemen van deze enzymen onderzocht in deze 10 patiënten. In onze studie zagen we geen

verschil in opname of blootstelling aan ivacaftor tussen beide groepen. De inname van pancreasenzymen veranderde de opname of de blootstelling aan ivacaftor niet in de CF patiënten met een slecht werkende alvleesklier. Op basis van onze resultaten kan het huidige doseringsadvies van tezacaftor/ivacafor dus onafhankelijk van de alvleesklierfunctie gehandhaafd worden.

Hoofdstuk 5:
Kunnen mensen met CF na een nier- of levertransplantatie veilig behandeld worden met elexacaftor/tezacaftor/ivacaftor?

Sinds begin 2022 wordt de zogenaamde triple therapie met elexacaftor/tezacaftor/ivacaftor (afgekort ETI) vergoed voor mensen met CF en tenminste 1 F508del mutatie van 12 jaar en ouder. Dit medicijn geeft een indrukwekkende verbetering in longfunctie en zweetchloride in de meeste patiënten. Het gebruik van ETI in mensen met CF na een orgaantransplantatie is omstreden doordat zij het middel tacrolimus, een anti-afstotings medicijn, gebruiken. De medicijn interactie tussen ivacaftor en tacrolimus zou kunnen leiden tot verhoogde blootstelling aan tacrolimus en daardoor bijwerkingen kunnen veroorzaken. Met ons onderzoek wilden wij beter inzicht krijgen in de veiligheid en voordelen van behandeling met ETI en tacrolimus. Wij hebben ervoor gekozen ons te richten op CF patiënten na een nier- of levertransplantatie aangezien ETI een indrukwekkende stijging in longfunctie liet zien in CF patiënten zonder transplantatie en wij hetzelfde effect verwachten bij deze groep patiënten. 5 patiënten werden geïncludeerd waarvan 3 na nier- en 2 na levertransplantatie. Tacrolimus spiegels werden bepaald gedurende 2 weken voor het starten van ETI en 4 weken erna. De spiegels van ETI en het klinisch effect van de behandeling werden onderzocht. Na 4 weken behandeling met ETI was de blootstelling aan tacrolimus een factor 1,79 hoger dan voor start van de ETI. In totaal werd de dosering tacrolimus 5 keer aangepast in 4 patiënten om zo de tacrolimus spiegel goed te houden. Er werden geen bijwerkingen gemeld. ETI had in alle patiënten een erg goed effect. De spiegels van ETI verschilden sterk tussen patiënten. Deze variatie werd ook al in andere onderzoeken gevonden. Ons onderzoek laat zien dat er inderdaad een medicatie interactie optreedt tussen ETI en tacrolimus. Gezien het duidelijke klinische effect van ETI adviseren wij behandelaren om behandeling met ETI te overwegen in patiënten na een nier- of levertransplantatie en de tacrolimus spiegels goed op te volgen om tijdig de dosering aan te kunnen passen. De veiligheid van een langdurige behandeling moet verder onderzocht worden. Of patiënten na longtransplantatie ook veilig behandeld kunnen worden met ETI en of de nadelen opwegen tegen de voordelen wordt momenteel onderzocht in een grote studie in Nederland.

Hoofdstuk 6:
Wat is het effect van behandeling met lumacaftor/ivacaftor in mensen met CF en de "Nederlandse" A455E mutatie?

Eind 2017 werd lumacaftor/ivacaftor (ook wel orkambi) vergoed voor patiënten met 2 keer de zogenaamde F508del mutatie, de meest voorkomende mutatie wereldwijd. Door onderzoeken in het laboratorium waren er aanwijzingen dat dit medicijn ook bij mensen met een A455E mutatie zou werken. Deze mutatie is erg zeldzaam maar komt in Nederland bij ruim 4% van de CF patiënten voor. In dit onderzoek hebben we gekeken of patiënten met deze mutatie verbeterden met deze behandeling gedurende 8 weken. We zagen een duidelijke verbetering van het zoutgehalte in het zweet. De longfunctie en kwaliteit van leven waren niet duidelijk veranderd na 8 weken behandeling. We weten dat de zweettest heel snel kan verbeteren na starten van behandeling terwijl dit voor andere uitkomsten zoals longfunctie vaak langer duurt. Omdat er aanwijzingen waren dat het medicijn een positief effect had mochten alle deelnemers in de studie het medicijn blijven gebruiken. Uit de metingen na ongeveer een half jaar bleek dat ook de longfunctie was verbeterd. Orkambi werd uiteindelijk ook voor patiënten met deze mutatie vergoed.

Hoofdstuk 7:
Een 7 jarig meisje met CF met borstvorming. Te veel ivacaftor?

Behandeling met het middel ivacaftor, een potentiator die de opening van het chloride kanaal verbetert, zonder corrector is zeer effectief gebleken in patiënten met een specifieke mutatie waarbij er wel voldoende chloride kanaaltjes zijn maar deze niet goed openen (zogenaamde gating mutaties). In dit hoofdstuk wordt een meisje beschreven die behandeld wordt met ivacaftor en borstvorming krijgt op 7 jarige leeftijd. Het is zeer aannemelijk dat dit door ivacaftor komt aangezien deze bijwerking volledig verdween na stoppen van het medicijn en dit al eerder beschreven is op latere leeftijd en bij mannen die ivacaftor gebruiken. Uiteindelijk werd besloten de ivacaftor dosering te verlagen. Dit had een goed effect en er traden geen bijwerkingen op. Dit illustreert het belang van inzicht in de beste dosering voor een goed effect maar zonder bijwerkingen (ook wel de therapeutische breedte genoemd). In een kleine groep patiënten werd de relatie tussen de hoeveelheid medicijn in het bloed en het effect zoals longfunctie en zweetchloride onderzocht. Deze relatie kon niet worden aangetoond. Het moet verder onderzocht worden of patiënten veilig behandeld kunnen worden met een lagere dosering ivacaftor.

Hoofdstuk 8:
Een overzicht van bijwerkingen door CF medicijnen – Kunnen we deze voorkomen?

CF is een complexe ziekte die schade geeft aan meerdere organen. Verbeterde behandeling van CF, mede door CFTR modulatoren, heeft ervoor gezorgd dat mensen met CF steeds ouder worden. Gedurende hun leven gebruiken zij veel medicijnen waarvan een groot aantal chronisch. Iedere behandeling kan bijwerkingen veroorzaken, soms direct en soms op langere termijn. In dit hoofdstuk geven we een overzicht van veel gebruikte medicijnen in de CF behandeling, interactie tussen verschillende medicijnen, de bijwerkingen die veel voorkomen en hoe we deze zouden kunnen voorkomen. Als behandelaren zich hier goed van bewust zijn worden bijwerkingen hopelijk eerder herkend en wellicht voorkomen.

Hoofdstuk 9:
Conclusie

Recente ontwikkelingen in de behandeling van CF met zogenaamde CFTR modulatoren leveren nieuwe inzichten op maar leiden ook tot nieuwe vragen welke opgehelderd moeten worden om ieder individu met CF zo effectief en veilig mogelijk te kunnen behandelen. Hiervoor is kennis van de dosis-blootstelling-effect relatie en inzicht in medicatie interacties van groot belang.

De onderzoeken in dit proefschrift hebben laten zien dat de variatie in blootstelling aan CFTR modulatoren tussen mensen met CF bij dezelfde toegediende dosering erg groot is. Waarom dit zo is is nog onduidelijk. Wij dachten dat de functie van de alvleesklier hierbij een rol zou kunnen spelen. We hebben echter laten zien dat een gestoorde vetvertering door een slecht werkende alvleesklier de blootstelling aan het medicijn ivacaftor niet significant veranderde. Of andere eigenschappen van mensen met CF het verschil aan blootstelling wel kunnen verklaren moet verder worden onderzocht. Ook hebben we aangetoond dat er relevante interacties zijn tussen ivacaftor en andere medicijnen die vaak gebruikt worden in de CF behandeling. Door hier goed op te letten bijvoorbeeld door bloedspiegels te meten kunnen patiënten behandeld worden met voor hen belangrijke medicijnen, zoals tacrolimus en CFTR modulatoren. Om in de toekomst bloedspiegel bepalingen van CFTR modulatoren te kunnen gebruiken om de beste dosering voor een individuele patiënt vast te stellen is een beter inzicht nodig in de relatie tussen de blootstelling en het effect van de modulator. Aangezien ETI de meest voorgeschreven CFTR modulator behandeling is, verwachten we meer inzicht in deze relatie te krijgen door bij patiënten die ETI gebruiken de bloedspiegels en het effect van de behandeling te meten.

Appendices

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List of publications
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