university of groningen / faculty of science and engineering

Patient-Derived Organoids for Personalized Medicine in Cystic Fibrosis

Literature review on the potential of Patient-Derived Organoids in developing personalized treatments for Cystic Fibrosis.

08/07/2024

Bachelor's Thesis Life Sciences

Leanne Speelman (S4945115)
BSc Life Science and Technology

Supervised by Prof. Dr. R.P. Coppes

Summary

Cystic fibrosis (CF) is a life-threatening disease that is caused by mutations in the cystic fibrosis transmembrane regulator (CFTR) gene. Although in-depth knowledge of the molecular basis of CF and the development of CFTR modulator treatments have already majorly improved CF treatment, not all CF patients are eligible for effective CF treatment yet. This is due to the large variety of clinical phenotypes resulting from over 700 identified disease-causing CFTR mutations. Currently, about 10 to 20% of CF patients are non-eligible for highly effective modulator treatments, as these patients carry rare CFTR mutations and have therefore often been excluded from clinical trials. To increase the accessibility to CFTR-restoring therapies for these patients, personalized medicine approaches could provide an outcome. Patient-derived organoids (PDOs) have shown to be a promising tool for performing personalized medicine approaches in a cost-efficient and patient-friendly manner. The forskolin-induced swelling assay developed in 2013 can reliably assess CFTR function in patient-derived intestinal organoids and therefore paved the way for organoid usage in CF treatment. Currently, PDOs have shown to possess great potential in identifying efficient therapies for individual CF patients. Moreover, organoids can be used in the early developmental stages of new CF therapies specified for subgroups of CF patients with rare mutations. Living biobanks that store these organoids can further expand the possibilities of PDO usage in CF research and personalized treatments. This thesis provides an overview of the current therapies available in Cystic fibrosis, the developments in patient-derived organoids, and the usage and potential of patient-derived organoids in personalized medicine approaches in CF.

Table of Contents

Introduction

Cystic fibrosis (CF) is one of the most common inherited life-threatening diseases, affecting about 1:6000 newborns in the Netherlands (Dankert-Roelse et al., 2019) and by estimation more than 160,000 patients worldwide (Guo et al., 2022). The autosomal recessive disease is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene, which encodes an epithelial anion channel (Riordan et al., 1989). This channel mediates chloride and bicarbonate transport across epithelia of the airways and other organs (Ramalho et al., 2023). Better understanding of the molecular basis of CF and the development of CFTR modulator treatments have already majorly improved CF treatment, increasing the estimated median age of survival to just below 50 years in developed countries (Scotet et al., 2020). However, room for improvement in the field of CF treatment remains. More than 2000 different CFTR mutations have been identified, of which more than 700 are confirmed as disease-causing (http://cftr2.org). This large variety of disease-causing mutations leads to a wide spectrum of clinical CF phenotypes. By far the most common mutation is the F508del mutation, found in approximately 70% of CF chromosomes and present in ~85% of people with CF (PwCF) on at least one allele (http://cftr2.org). For this 85% of PwCF, the highly effective modulator treatments (HEMTs), capable of correcting the basic CFTR protein defect, have revolutionized treatment (De Poel et al., 2023). However, 10 to 20% of PwCF are not eligible for the available HEMTs, mainly because many of them carry rare or ultra-rare mutations. The responsiveness of these PwCF to the modulator treatments is often still unknown, as patients with these rare mutations have frequently been excluded from clinical trials due to the low number of people carrying these specific mutations (Ramalho et al., 2023). Moreover, even patients with identical CFTR mutations may respond differently to treatments based on environmental influences, their demographics, and their individual genetic background (Ramalho et al., 2023). A large unmet need to identify new and affordable treatments for PwCF that are non-eligible or non-responsive for HEMTs is therefore present (De Poel et al., 2023). Personalized medicine approaches could be used to address this unmet need. Taking into account patients' genotypes when choosing their appropriate treatments seems a necessity for the establishment of therapies for all individuals with CF. Identifying which patients might benefit from certain treatments, like HEMTs, is however a time-consuming, costly, and challenging process for individuals with rare CF mutations (Dekkers et al., 2016). A possible solution to make this process more efficient, would be the use of patient-derived organoids (PDOs). These mini-organs derived from patient material replicate the structure and function of the patient's original organ or tissue and therefore can aid in finding the ideal treatment for a specific patient (Bartfeld & Clevers, 2014). PDOs might therefore provide a personalized medicine approach to increase access to treatments for all CF patients and optimize the risk-benefit and cost-effectiveness of treatments (Berkers et al., 2019). This thesis will focus on the use of PDOs in personalized medicine approaches for cystic fibrosis and answers the research question: 'What is the potential of patient-derived organoids in the development of personalized treatments in Cystic Fibrosis?'.

CF pathophysiology and current therapeutics

More than 85 years ago, Cystic fibrosis was identified as a disease for the first time by Dorothy Anderson (Anderson, 1938). Back in that time, the median life expectancy for CF patients was only a few months. Since the identification of the disease, a lot has changed in the field of CF. The identification and cloning of the CFTR gene in 1989 marked an important landmark in the history of CF, as it enabled a better understanding of the pathophysiology of the disease, improved the diagnosis and management of CF patients and their families, and paved the way for mutation-specific therapies (Farell et al., 2020; Riordan et al., 1989). The development of CFTR modulator treatments, of which the first, Ivacaftor, was approved in 2012, revolutionized CF treatments even further (Ramalho et al., 2023). Nowadays, the number of adult patients surpasses that of children patients, and the median life expectancy has increased to just below 50 years (Scotet et al., 2020).

Causative of CF are mutations in the CFTR gene, leading to a dysfunction of the CFTR protein. This protein is mainly expressed in the lung and the pancreas, but also in sweat glands, the intestine, the liver, nasal mucosa, salivary glands, and the reproductive tract (López-Valdez et al., 2021). Under normal conditions, CFTR functions as a cyclic adenosine monophosphate(cAMP)-dependent, phosphorylation-activated anion channel in the cell membrane of epithelial cells, that opens towards the extracellular side to allow the transport of specific anions as chloride (Cl-) and bicarbonate (HCO3-) (Gentzsch & Mall, 2018; López-Valdez et al., 2021). CFTR regulates the local pH, mainly in the airways, by allowing the outflow of Cl- and HCO3-, and by causing the epithelial sodium channel (ENaC) to transport sodium (Na+) into the cell (López-Valdez et al., 2021) (*Figure 1*). This way, the airway surface fluid (ASF) maintains a pH of around 7.0 (López-Valdez et al., 2021).

In CF, the dysfunction of CFTR leads to a lack of effective Cl- and HCO3- secretion into the extracellular space. This causes the pH of the ASF to be eight times as acidic (López-Valdez et al., 2021). Moreover, the loss of Cl- disrupts the balance of osmotic pressure and electroneutrality, leading to excessive Na+ and water absorption as visualized in *Figure 1* (López-Valdez et al., 2021). Acidification and dehydration of the ASF increase the viscosity of mucus and provide difficult clearance of mucus in the airways (López-Valdez et al., 2021). This is often followed by a progressive muco-obstructive lung disease, in which the airways are blocked by thick mucus, leading to ongoing bacterial infections and inflammations which make up the main causes of illness and death in CF (Gentzsch & Mall, 2018; Mall & Hartl, 2014). Dehydration and acidification of mucosal surfaces are also present in the other organs in which CFTR functions, creating thick and sticky mucus that obstructs luminal compartments and ducts in various organs, causing their dysfunction (Gentzsch & Mall, 2018).

Although the above-mentioned information gives a general view of CF pathophysiology, the large variety in CF-causing CFTR mutations yields a wide spectrum of CF phenotypes. Different mutations affect the CFTR protein in different ways. Therefore CF-causing mutations are grouped into six categories based on their effects on the CFTR protein as vizualized in *Figure 1* (Gentzsch & Mall, 2018). Class Ⅰ mutations result in no CFTR protein production. Class Ⅱ

mutations, which include the common F508del mutation, cause the CFTR protein to be misfolded at the endoplasmic reticulum, leading to degradation by the proteasome. Class Ⅲ mutations impair the opening of the CFTR channel and class Ⅳ mutations show reduced conductance: a defect in the ion transport through the CFTR protein causes decreased outflow of ions from the cell. In class Ⅴ mutations the quantity of CFTR mRNA or protein, or both, is reduced, decreasing ion outflow. Lastly, class Ⅵ mutations cause decreased CFTR stability, lowering the CFTR protein's half-life, and thus accelerating CFTR turnover (López-Valdez et al., 2021).

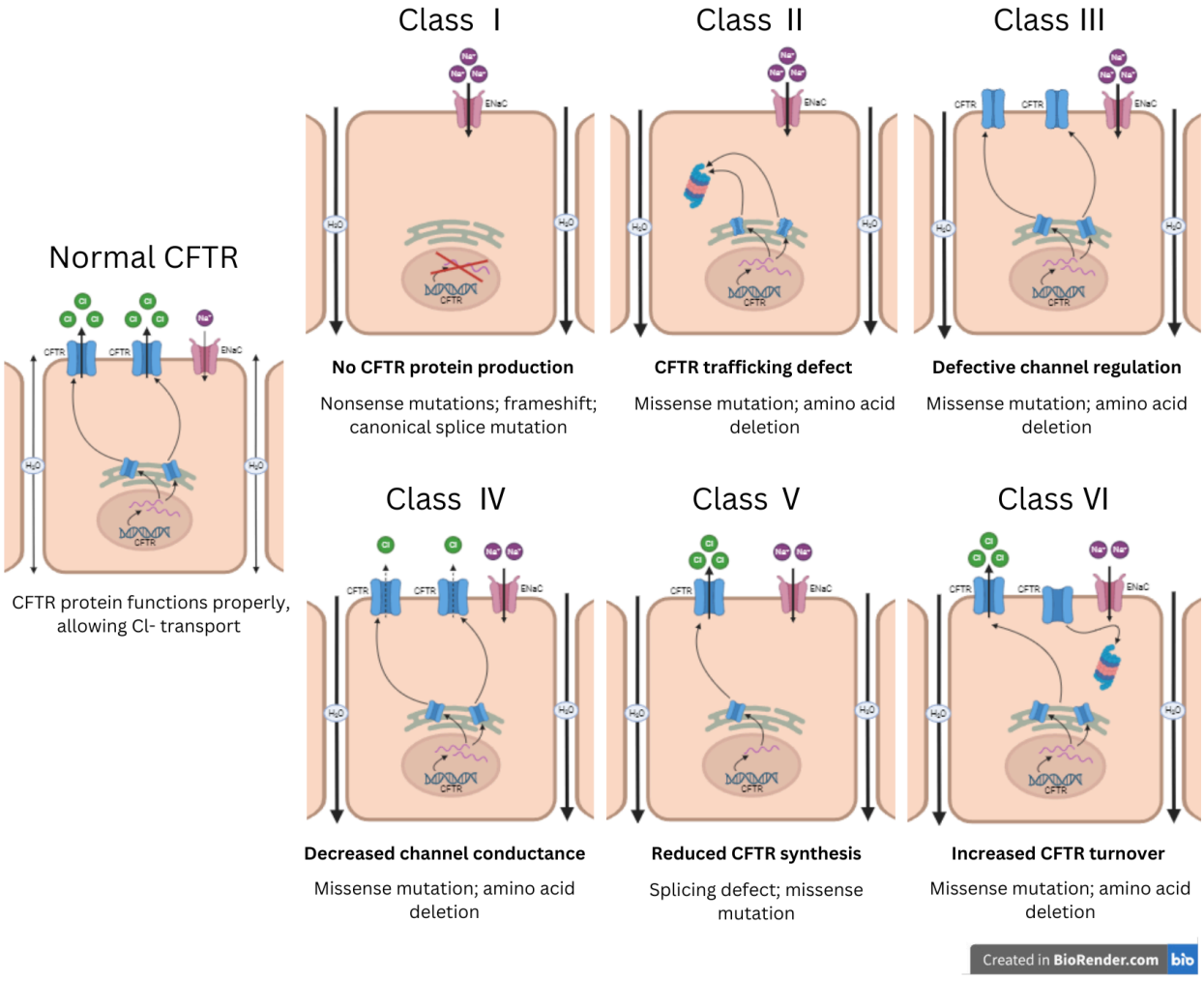

Figure 1: Classification of CF-causing CFTR mutations, based on their effect on the CFTR protein.
CFTR mutations that are causative of CF are grouped into six classes based on their effect on the CFTR protein. Class Ⅰ mutations result in no protein production; Class Ⅱ mutations create a misfolded CFTR protein that gets degraded by the proteasome; Class Ⅲ mutations impair CFTR channel opening; Class Ⅳ mutations decrease ion transport through the CFTR channels; Class Ⅴ mutations decrease CFTR mRNA and/or protein synthesis; Class Ⅵ mutations increase CFTR turnover. Illustrated are the CFTR defects and their consequences in epithelial airway cells, per mutation class. Moreover, the kind of mutations within each class are mentioned. The figure is made based on information from Elborn (2016), Gentzsch & Mall (2018) and López-Valdez et al. (2021).

A first line of CF treatment is formed by the symptomatic treatments. These treatments have for a long time been the major form of CF treatment, and are also called standard treamtents (De Boeck & Amaral, 2016). They include mucolytics that dissolve thick mucus, antibiotics to treat or prevent infections, and anti-inflammatory agents that dampen chronic inflammation (De Boeck & Amaral, 2016). Although these treatments are effective in reducing CF symptoms and enable most patients to live to adulthood, they do not address the underlying cause of the disease: the molecular defects of the CFTR protein. Therefore, most attention is now focused on CF modulator treatments, particularly the highly effective ones known as HEMTs, that are able to correct the basic CFTR protein defect. These still quite recently developed modulator treatments have majorly improved CF treatment, but are not effective enough yet to function as a stand-alone treatment (De Boeck & Amaral, 2016). Therefore, symptomatic treatments are still used in combination with modulator treatments, and will remain an important part of CF therapy until therapies are found or refined that are able to eliminate the root cause of CF and entirely prevent the display of symptoms (De Boeck & Amaral, 2016).

HEMTs have been introduced for PwCF carrying specific mutations since 2012. They can be divided into potentiators, that increase CFTR channel activity, and correctors, that restore CFTR F508del protein folding and enhance its transport to the cell membrane (Ramalho et al., 2023). The first approved HEMT, Ivacaftor (VX-770), is a potentiator drug that improves CFTR function in patients with gating mutations (Class Ⅲ mutations) by increasing the frequency of CFTR channel opening and its ion conductance (Eckford et al., 2012). The other current HEMTs, Lumacaftor (VX-809), Tezacaftor (VX-661), and Elexacaftor (VX-445), are all corrector drugs. Their exact functioning is not known yet, but it is suspected that these drugs repair the unusual assembly of the full-length protein, improve protein folding in the endoplasmic reticulum, and improve the trafficking and stability of the protein to the cell membrane (Okiyoneda et al., 2013).

Ivacaftor (VX-770; Kalydeco) was first only approved for PwCF of six years or older carrying at least one G551D mutation but was rapidly expanded to other class Ⅲ mutations and patients of younger age (Regard et al., 2022). The drug showed great improvement in respiratory symptoms and lung function for patients carrying one of these mutations (Regard et al., 2022). In F508del homozygous patients, Ivacaftor however showed no effect on lung function (Flume et al., 2012). The promising results of Ivacaftor, combined with the need to treat more PwCF, resulted in the development of Lumacaftor and Tezacaftor used in combination with Ivacaftor (respectively VX-770+VX-809; Orkambi and VX-770+VX-661; Symdeco/Symkevi) to target F508del mutations. Orkambi was approved in 2016 for patients homozygous for the F508del mutation, and Symdeco/Symkevi was approved in 2020 for PwCF carrying at least one F508del mutation and selected mutations that show partial CFTR functionality (Regard et al., 2022). The improvement in respiratory parameters using these drugs was however modest compared to Ivacaftor in PwCF carrying class Ⅲ mutations (Regard et al., 2022). Moreover, Orkambi and Symdeco/Symkevi were ineffective in patients carrying one F508del mutation and one minimal function mutation, such as a class Ⅰ mutation (Munck et al., 2020). Therefore triple-combination therapy that targets different CFTR sites was developed, consisting of Ivacaftor, Tezacaftor, and Elexacaftor (VX-770+VX-661+VX-445; Trikafta or Kaftrio). This triple-combination treatment was first approved in 2020 for PwCF aged 12 or older who were

F508del homozygous or had one F508del mutation and a minimal function mutation (Regard et al., 2022). Approval was extended to patients with one F508del and one gating (class Ⅲ) or partial function mutation by 2021. Clinical trials showed that this triple-combination therapy yields the greatest clinical improvement observed with HEMTs (Regard et al., 2022). A summary from Regard et al. (2023) of the history of HEMT approval in Europe is shown in *Table 1*.

Table 1: Evolution in CFTR modulators approval and indications in Europe obtained from Regard et al. (2023)

Modulator	Approval (year)	Approved (ages)	Target mutations
Ivacaftor	2012	≥6 years	At least one copy of the G551D mutation
	2014	≥6 years	At least one gating (class III) mutation: G551D, G1244E, G1349D, G178R, G551S, S1251N, S1255P, S549N or S549R
	2016	≥2 years	
	2019	≥1 year	
	2020	≥6 months	
	2021	≥4 months	At least one gating (class III) mutation: G1244E, G1349D, G178R, G551D, S1251N, S1255P, S549N, S549R or G970R or at least one copy of the R117H mutation
Lumacaftor + ivacaftor	2016	≥12 years	Two copies of the F508del mutation
	2018	≥6 years	
	2019	≥2 years	
Tezacaftor + ivacaftor	2020	≥12 years	Two copies of the F508del mutation or one copy of the F508del mutation AND one of the following mutations: P67L, R117C, L206W, R352Q, A455E, D579G, 711 + 3A→G, S945L, S977F, R1070W, D1152H, 2789 + 5G→A, 3272 26A→G, 3849 + 10kbC→T.
	2021	≥6 years	
Elexacaftor + tezacaftor + ivacaftor	2020	≥12 years	Two copies of the F508del mutation or one copy of the F508del mutation and one minimal function mutation
	2021	≥12 years	At least one F508del mutation

The existing CF modulator treatments are specific to certain mutations or mutation classes. This CFTR genotype-based classification for drug prescription provides a problem for individuals with rare CFTR mutations who have not been included in clinical trials. In the United States, in vitro data has led the Food and Drug Administration (FDA) to expand approval of HEMTs to patients with an increasing number of mutations, now approving triple-combination therapy for PwCF who have one of 178 different mutations (Regard et al., 2023). Still, a significant proportion of CF patients is not eligible for HEMTs. To expand these treatments to an even larger group of CF patients, CFTR mutations could be categorized based on in vitro responses to CFTR modulators. In Europe, this strategy has not been confirmed to extend the acces and approval of triple-combination therapy. Here, the therapy is still only approved for PwCF aged 6 years or older, carrying at least one F508del mutation (Regard et al., 2023).

Corrective treatments that are non-specific to mutation type, and thus applicable to every CF patient, such as gene therapy or activation of other ion channels that could bypass CFTR are being examined, but these treatments are still in the early stages of development (De Boeck & Amaral, 2016). To be able to treat a greater number of CF patients shortly, personalized medicine approaches seem necessary.

Patient-derived organoids

One of the major recent breakthroughs in the field of personalized medicine includes the development of organoids. The term ‘organoid’ literally means ‘resembling an organ’ (Lancaster & Knoblich, 2014). Organoids are three-dimensional cellular structures derived from stem cells and grown in vitro (Bose et al., 2021). They are defined by three characteristics: they should contain multiple cell types of the organ it models, display some function specific to the organ, and self-organize in a manner similar to the establishment of the organ's characteristic organization during development (Lancaster & Knoblich, 2014). In the synthesis of organoids, the self-renewing and multipotent characteristics of stem cells are used. Any stem cell should be able to self-renew and produce differentiated daughter cells, ideally developing into tissue- or organ-like structures, when given the right culture conditions (Bartfeld & Clevers, 2014)

As the name suggests, patient-derived organoids are obtained using tissue derived directly from patients. PDOs can be made from either induced pluripotent stem cells (iPSCs), which are somatic cells reprogrammed into pluripotent cells by the addition of several transcription factors (Takahashi et al., 2007), or organ-specific adult stem cells (ASCs) (Bose et al., 2021). Synthesis of both types of organoids follows the same basic principle. The organoids develop in a similar way to the process of organs acquiring their unique structures. Organoids organize themselves by cells committing to specific functions and sorting into structures. This process requires the activation of multiple signaling pathways, influenced by internal cell components and external factors like the extracellular matrix (ECM) and growth media (Corrò et al., 2020). The most used material to resemble the ECM is Matrigel, a varied, gel-like protein mixture secreted by Engelbreth-Holm-Swarm mouse sarcoma cells (Corrò et al., 2020; Kleinman et al., 1986). Matrigel mimics the natural ECM, providing a scaffold for cell attachment and establishing a 3D culture environment resembling *in vivo* conditions (Corrò et al., 2020).

ASC-derived PDOs are made directly from adult tissue, either from single ASC or ASC-containing tissue units. The development of organoids from these ASCs is supported by a variety of growth factors present in the culture media, that mimic the signaling control under normal tissue homeostasis (Corrò et al., 2020). ASC-derived organoids can easily be made from patient-specific tissue for disease modeling and personalized medicine (Corrò et al., 2020). Synthesis of iPSC-derived organoids, on the other hand, entails stepwise differentiation protocols using multiple growth factors or inhibitors resembling the developmental cues during early embryonic development and organogenesis (Corrò et al., 2020). iPSC-derived organoids are especially useful for studying early-stage embryonic development, as here primary human material is limited, and for tissues with little self-renewal capacity, such as parts of the central nervous system, heart muscle, and kidney glomeruli (Bose et al., 2021; Corrò et al., 2020) Moreover, the pluripotent properties of iPSCs facilitate the development of organoids from all three germ layers, suggesting that it is possible to develop organoids for the majority of organs (Bartfeld & Clevers, 2014).

Before the establishment of organoids, immortalized cell lines and genetically engineered mouse models (GEMMs) formed the foundation of medical scientific research (Bose et al.,

2021). Creating immortalized cell lines from tissues is however an inefficient process and often causes significant genetic changes when adapting to 2D culture, and the mutations induced in GEMMs to generate disease phenotypes do not grasp the diversity of human disease phenotypes, and (Bose et al., 2021). Moreover, GEMMs are work-, time- and cost-intensive, and are seen as poor predictors of actual clinical success (Bose et al., 2021). Since the first successful establishment of ASC-derived organoids by Sato et al. in 2009, namely the development of intestinal organoids from Lgr5-expressing ASCs, organoids have rapidly developed as a frontier tool for disease modeling, being a relatively cost-efficient and convenient method (Bose et al., 2021). PDOs can be generated relatively easily once optimal culture conditions are established, and limited primary tissue, such as needle biopsies, is needed (Currò et al., 2020). As PDOs replicate the structure and function of a specific patient's original tissue or organ, they have the potential to identify the ideal treatment for specific patients (Bartfeld & Clevers, 2014). PDOs thus show great potential for application in personalized medicine by helping to predict for whom specific therapies will be most beneficial, and by functioning as drug screening and drug safety tests. Storing PDOs in living biobanks expands this potential, as PDO libraries can facilitate the identification of effective targeted therapies for specific disease subsets of patients, by selecting PDOs of this subtype for drug-sensitivity screening (Bose et al., 2021). As organoids can be made from nearly every patient, either from iPSCs or tissue biopsies containing ASCs, it allows for the study of rare mutations (Bartfeld & Clevers, 2014). This makes PDOs especially interesting for personalized medicine in CF, where patients with rare and ultra-rare mutations are often non-eligible for efficient therapies. In the future PDOs could even play a role in regenerative medicine. As organoids can be derived from small amounts of donor cells, they could provide tissue for transplantation, reducing the shortage of transplantation material (Bartfeld & Clevers, 2014).

PDOs in personalized medicine in CF

After the first development of ASC-derived intestinal organoids by Sato et al. in 2009, the implementation of PDO usage in CF treatment followed quite quickly. By 2013 Dekkers et al. generated intestinal organoids from rectal biopsies of human CF patients carrying the F508del mutation, using the human intestinal organoid culture conditions developed by Sato et al. in 2011. Dekkers et al. (2013) developed an assay that was able to quantify CFTR function, the Forskolin-induced swelling (FIS) assay: a robust and relatively simple assay that could facilitate diagnosis, functional studies, drug development, and personalized medicine approaches in CF. This newly developed assay quickly showed its potential. In 2015 the first CF patient was treated based on the information from drug-screening tests performed on the patient's own tissue, cultured in the form of intestinal organoids, using the FIS assay (Chakradhar, 2017). Treating this patient was difficult before, as he carried a rare G1249R mutation on one of his alleles and therefore was not eligible for the then recently approved drug Ivacaftor. As this drug did perform very well on the patient's intestinal organoid, his doctor still decided to prescribe him the treatment, in this way saving the boy's life (Chakradhar, 2017).

The intestinal organoid culture conditions developed by Sato et al. (2011) enable intestinal ASCs to grow into organoids containing crypt-like structures and an internal lumen lined with differentiated cells. These organoids express CFTR just like in vivo organs do: CFTR is mainly expressed at the apical membrane of the crypt cells where it drives anion secretion and mediates fluid secretion upon activation (Dekkers et al., 2013). The natural compound forskolin raises intracellular cAMP, thereby activating CFTR and its associated anion transport, leading to fluid secretion into the lumen of the intestinal organoid (Dekkers et al., 2013). The addition of forskolin to intestinal organoids therefore results in rapid organoid swelling, visible by the rapid growth of the lumen and total organoid surface area (Dekkers et al., 2013). This FIS is quantifiable by live-cell confocal microscopic readout using automated fluorescent image analysis (Dekkers et al., 2013). Removal of forksolin, or chemical inhibition of CFTR, reverses the organoid's swelling. Moreover, organoids derived from healthy individuals show strong FIS, while organoids derived from patients carrying mutations associated with mild CF show reduced FIS, and organoids from subjects with severe CF genotypes show much less FIS (Dekkers et al., 2013). Combining this data, Dekkers et al. (2013) showed that their FIS assay is CFTR-dependent and can accurately measure CFTR function in intestinal organoids. Furthermore, it was shown that functional restoration of CFTR F508del in human intestinal organoids, either by low temperature or the CFTR modulators Ivacaftor and Lumacaftor, could increase FIS, indicating that the FIS assay can reliably measure correction or potentiation of F508del CFTR. The results also demonstrated that the potency of CFTR modulators varies greatly between organoids from PwCF, even in CFTR F508del homozygous organoids. This raised the possibility that their in vitro assay can predict the drug responsiveness of individuals in vivo.

To test whether this hypothesis was true, Dekkers et al. again studied CFTR function and responses to Ivacaftor and Lumacaftor in patient-derived intestinal organoids (PDIOs) from CF patients with a wide variety of CFTR mutations in 2016. This time comparing their results based on FIS assays of PDIOs to published data from clinical trials with both CFTR modulators. This study indeed confirmed that in vitro drug responses in rectal organoids correlate with the published clinical trial data, allowing the prediction of drug responsiveness for PwCF carrying rare or ultra-rare CFTR mutations based on preclinical data. A study by De Winter-De Groot et al. (2018) even suggested that FIS measurements can quantify CFTR function more precisely than the standard in vivo biomarker sweat chloride concentration.

The FIS assay in PDIOs thus provides a personalized medicine approach for identifying patients who might benefit from CFTR modulator treatments, independent of their CFTR mutations (Dekkers et al., 2016). An ideal therapeutic model for this would be to screen the effectiveness of available HEMTs and other CFTR-restoring drugs on a patient’s intestinal organoid immediately after CF diagnosis. In this way, treatment could be optimized before disease onset even occurs (Dekkers et al., 2013). The usage of PDOs in personalized medicine might also facilitate the development and approval of new drugs specific to subgroups of CF patients with rare mutations in a way that limits the economic risks normally associated with drug research (Dekkers et al., 2013). Organoid cultures seem superior in generating large data sets from individual patients, compared to other, earlier techniques that can predict individual drug

responses, such as ex vivo rectal biopsies and primary airway tissue culture models. Determining CFTR function using the FIS assay in organoids is a relatively fast, easy, and robust process, as organoids self-differentiate and can be cultured in 96-well plates (Dekkers et al., 2013). This enables the measurement of up to 80 organoids per well and up to 96 conditions per experiment (Dekkers et al., 2013). Moreover, living organoids biobanks can be generated by storing CF patients' organoids in liquid nitrogen (Dekkers et al., 2013). These biobanks can be used to study cellular factors associated with specific clinical phenotypes and to analyze the effect of newly developed drugs on individual patients using organoids that have already been acquired in the past (Dekkers et al., 2013)

A large current study that generates such a biobank is the European Human Individualized Treatment for CF (HIT-CF) Organoid Study (*Figure 2*). This multi-center study, funded by the European Union's Horizon2020 program, generated PDIOs from rectal biopsies of approximately 500 CF patients from 17 different countries of the European Union (Van Mourik et al., 2020). The examined patients all carry rare CFTR mutations and are currently excluded from classical CFTR-modulator trials. Their organoids were sent to central laboratories where several novel CFTR-modulating drugs were screened for efficiency using a FIS protocol that was fully standardized between the independent laboratories (Van Mourik et al., 2020). Individuals whose organoids responded to one of the treatments were asked to participate in follow-up clinical trials to evaluate the efficacy of the drugs, which is the stage the study is currently in. In this way, the HIT-CF study makes efforts to create a new pathway for access to CFTR-modulating drugs for patients with ultra-rare CFTR variants (Van Mourik et al., 2020). The organoids generated in this process were stored in a biobank, developing a library of PDIOs of about 500 CF patients with rare mutations that can be used for future research (Van Mourik et al., 2020). Upon the consent of patients for the long-term storage of their organoids, these can be used by academic laboratories worldwide (Van Mourik et al., 2020). Moreover, it could be explored to collaborate with pharmaceutical companies to screen new CFTR modulators in this large biobank (Van Mourik et al., 2020). As a last pathway, the HIT-CF program makes efforts to improve regulatory access and increase reimbursement of the drugs in the patient population, as the support of stakeholders such as regulatory agencies and insurance companies is needed to implement the use of organoids as a predictive tool for drug prescription in Europe. In this way, the program hopes to pave the way for personalized medicine in Europe, ensuring that patients with rare CFTR mutations can access effective therapies.

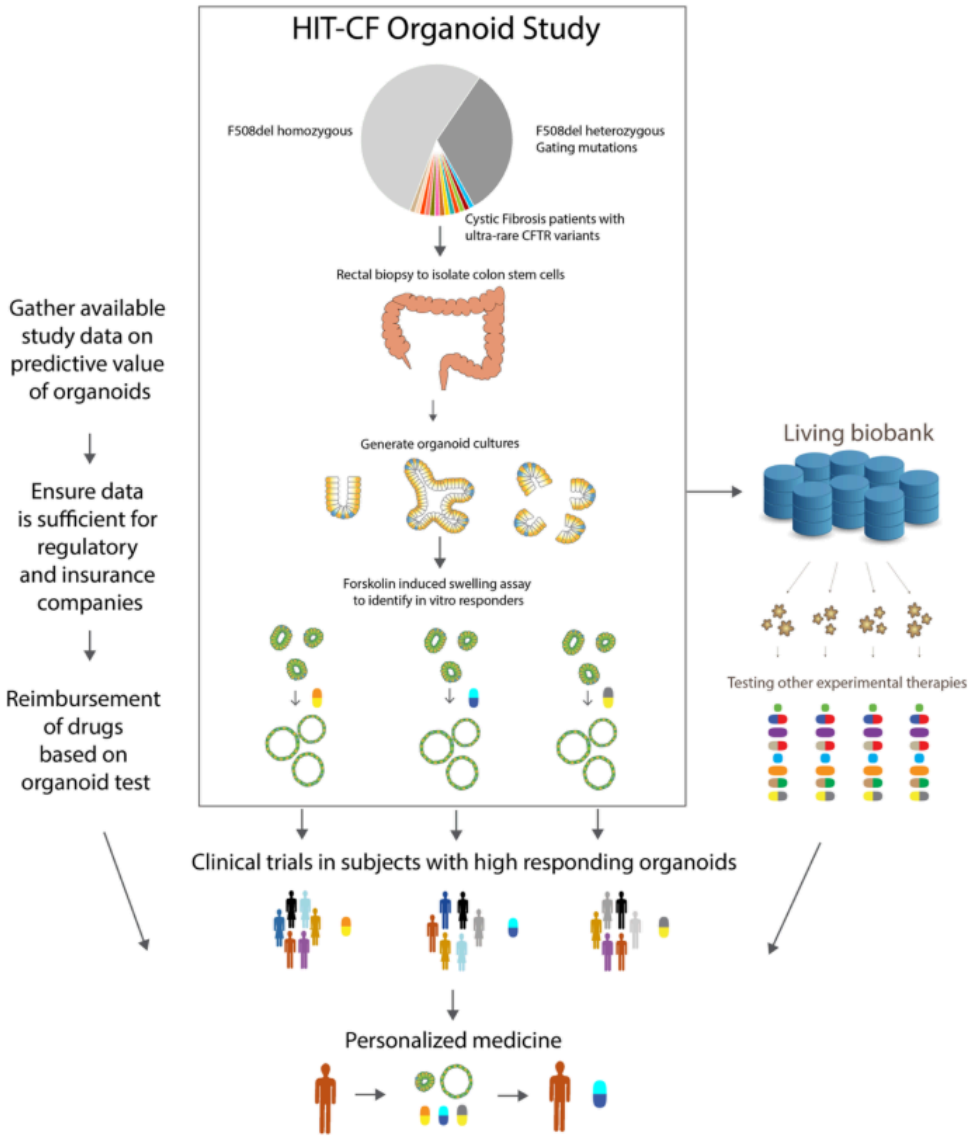

Figure 2: Visual overview of the HIT-CF Organoid Study obtained from Van Mourik et al. (2020).

In the United States, in vitro data is already used to expand CF treatment approval to increasing numbers of mutations. A recent study by De Poel et al. (2023) exemplified the possibility of using PDIOs in a high-throughput compound screening assay to enable personalized drug repurposing of FDA-approved drugs. Drug repurposing is the process of using approved drugs outside their original disease indication (Ashburn & Thor, 2004). The study of De Poel et al. (2023) established a new high-throughput screening version of the FIS assay in PDIOs, that allows testing compounds influencing CFTR function in a 384-well plate. Intestinal organoids are robust, scalable, and therefore very suitable for high-throughput screening (De Poel et al., 2023). In this study, 76 heterozygous F508del PDIOs were screened for 1400 FDA-approved drugs for improving CFTR function. The study found that PDE4 inhibitors, compounds that suppress cAMP degradation thereby increasing CFTR phosphorylation and function, are effective CFTR inducers in PDIOs where some functional CFTR is still present (De Poel et al., 2023). PDE4 inhibitors could therefore represent a new class of treatment compounds for cystic

fibrosis. Moreover, the study focused on improving theratyping of CF patients: ‘the matching of patients to beneficial compounds based on laboratory results of the patient-derived cells’ (De Poel et al., 2023). CFTR modulators (Ivacaftor and Lumacaftor) were found to be effective in multiple PDIOs of patients who were currently non-eligible for these treatments. As these CFTR modulators are already approved for specific CF-causing mutations, it would be only a matter of label extension to treat more CF patients with these effective drugs (De Poel et al., 2023). Additionally, the study’s results can help in theratyping unknown CFTR mutations into mutation categories, enabling better treatment.

Intestinal organoids are the most commonly used type of organoids in CF research, among others because they are easily obtained and cultured from rectal biopsies (a procedure which is painless and feasible to perform at all ages (Ramalho et al., 2023)), well-defined, and show robust and scalable characteristics that make them ideal for high-throughput studies (De Poel et al., 2023). Other types of organoids can however be used as well. Wong et al. (2012) for example developed lung organoids derived from iPSCs and showed that the function of these lung organoids was reduced when derived from CF patients carrying F508del mutations. The study of Amantgalim et al. (2022) shows another example of airway organoids used in CF, this time derived from 2D differentiated nasal epithelia. These organoids can also be used in a FIS assay and can yield new insights as the airways form the principal location of CF expression. In principle, organoids from all sorts of organs in which CFTR is expressed can be developed and used to study CF. Think of pancreatic organoids (Hennig et al., 2019) and liver organoids (Ogawa et al., 2021). PDOs can therefore provide a comprehensive tool for clinical improvements and personalized medicine in CF, as many different organs that are affected in CF can be modeled.

Challenges and limitations

Although PDOs show a lot of potential and have already contributed to the improvement of personalized medicine in CF, no method exists without its challenges and limitations. Not all PDOs are for example able to maintain all cell types of the tissue it models, and iPSC-derived organoids often mimic the fetal stages of development, indicating that some final differentiation steps are yet to be defined (Bartfeld & Clevers, 2017). Furthermore, a real organ, like the intestine, is more than just an inner epithelial layer and also consists of surrounding mesenchyme, muscles, nervous system, blood vessels, immune cells, and a microbiome (Bartfeld & Clevers., 2017). When using organoids to model organs, it should thus be considered that the organoid may not represent the complete functional organ.

In general, the use of organoids in drug screening only provides insight into the effect of these drugs on this specific organ model. The effects of the drug in a whole living individual can not be predicted based on these models. Even for the impact of a drug on a specific organ, organoids can not be viewed as a completely realistic model of the organ, as in the body other tissues and signaling pathways influence the function of the organ. Therefore new drugs can not be

developed solely based on their effects on PDOs and preclinical and clinical testing remains necessary.

The culture conditions for PDO synthesis, such as the materials used and the devices that generate consistent organoids, are not yet standardized (Bose et al., 2021). Therefore, results from different PDO studies can often not be compared to each other, as microenvironmental factors differ (Bose et al., 2021).

Although the usage of PDOs is generally seen as a cost-effective and relatively quick method compared to some other medical research methods, the costs and speed of PDO synthesis might in some cases still limit its use (Bose et al., 2021). The time necessary to develop PDOs is about 4 to 6 weeks, which might be too long for the previously mentioned ideal treatment procedure of using PDOs to screen the efficacy of available CF drugs immediately after CF diagnosis. Moreover, the cocktails of growth factors and Matrigel used for the culturing of organoids are expensive materials.

Apart from being an expensive material, Matrigel provides more limitations and downsides to the use of organoids. A possible future application of PDOs would be their use in regenerative medicine. For this however, the use of Matrigel in culturing organoids forms a bottleneck, as this material is obtained from mouse tumor lines, making the organoids not feasible for transplantation in humans (Bartfold & Clevers., 2017). Moreover, the use of Matrigel does not coincide with the growing movement in the scientific community toward more animal-free research. Although PDOs might look like an animal-friendly alternative for conventional animal-based personalized medicine approaches, like GEMMs, the use of Matrigel in culturing PDOs makes this method still far from animal-friendly or animal-free. Matrigel is obtained from mouse sarcoma cells (Kleinman et al., 1986). To produce Matrigel, tumors are introduced and grown in mice, whereafter they are harvested. Many mice are in this way used in the production of Matrigel, and therefore for the culturing of organoids. The limitations caused by the use of Matrigel for organoid culturing seem partly overcomeable, as at least for intestinal organoids, new matrices based on synthetic hydrogel networks have already been designed (Gjorevski et al., 2016). However, in daily practice, these synthetic matrices can not compete with Matrigel in their effectiveness of organoid culturing.

Discussion

Since the development of the first assay that could quantify CFTR function in CF PDOs, the FIS assay, in 2013, PDOs have shown to be a useful tool for personalized medicine in CF. They are used in drug screens to identify treatments for CF patients who are non-eligible for available treatment now, as is the case for many patients with rare or ultra-rare CFTR mutations. They can aid in theratyping unknown CFTR mutations into one of the mutation classes, enabling better treatment for patients carrying these mutations (De Poel et al., 2023). They can be used to extend the use of already approved CFTR modulator treatments for a larger number of CFTR mutations and to enable drug repurposing (De Poel et al., 2023). Moreover, PDOs show

potential for creating an ideal therapeutic model in which the effectiveness of available CFTR-restoring treatments could be screened directly after CF diagnosis to optimize therapy before the onset of the disease even occurs (Dekkers et al., 2013). PDOs might also be helpful in the development and approval of new drugs specific for CF patients with rare mutations. The use of PDOs in this process might limit the economic risks normally associated with drug research (Dekkers et al., 2013). Lastly, PDO biobanks show great potential for personalized medicine in CF. As PDOs can be stored in liquid nitrogen, these biobanks can be used to study specific clinical phenotypes and to examine the effects of newly developed drugs on various CF phenotypes and individual patients, using the organoids that were already acquired in the past (Dekkers et al., 2013). A large biobank, comprising around 500 intestinal organoids from CF patients with rare mutations all over Europe, is currently set up in the HIT-CF Organoid Study.

However, using PDOs in personalized medicine in CF does not come without challenges and limitations. Organoids can for example not predict the complete response of an organ, not to mention a complete living body, to a tested drug. Clinical trials are therefore still needed in the development of new CF drugs. Moreover, a need remains to develop standardized protocols for PDO development, as currently microenvironmental factors in the synthesis of organoids may differ too much between publications to compare PDO studies to one another (Bose et al., 2021). Ethical concerns about the use of organoids exist as well, as the use of Matrigel makes organoid culturing an animal-intensive process. It is therefore important to focus on the development of new animal-free matrices that do not limit the process of effective organoid culturing. Lastly, in Europe, the use of in vitro data to expand the approval of CFTR modulator drugs to a broader range of CFTR mutations is not yet validated (Regard et al., 2023).

In my opinion, these limitations however do not outweigh the advantages PDOs possess for personalized medicine. Quantifying CFTR function through the FIS assay in PDIOs is for example proven to be easy, fast, and robust compared to previously existing tools. These FIS assays can be used in high-throughput screening studies of up to 384-well plates, yielding way more and quicker results than obtained by conventional clinical trials. Moreover, the use of PDOs improved risk-benefit and cost-efficiency compared to drug development and personalized medicine approaches solely based on clinical trials. Most importantly, the use of PDOs allows for the inclusion of patients with rare and ultra-rare mutations that were previously excluded from clinical trials due to the low number of patients carrying these mutations.

To me, especially the large-scale HIT-CF Organoid Study seems to have a large potential to improve personalized medicine in CF. I could see the large biobank that is set up in this study significantly improving the accessibility of in vitro data of rare CFTR mutations for CF treatment developments in the future. Mostly, I really like that this study tries to tackle some of the current hurdles in PDO usage in CF, by for example standardizing the FIS assay protocol in the different participating laboratories, and by already trying to improve the regulatory access and to increase reimbursement of drugs for patients carrying rare mutations, as this is currently still very limited in Europe. In this way, this study will not only present their research findings but really makes an effort to improve the accessibility of CF therapies for patients with rare mutations.

Overall, I thus think the implementation of PDOs in the field of personalized medicine in CF shows a lot of potential, as it can provide a truly personalized method to improve accessibility to efficient treatments for individuals with CF. Although improvements still need to be made to optimize PDO usage, especially in making the process of organoid synthesis and culturing more animal-friendly, I believe PDOs can provide great advancements in moving towards a treatment approach in which every CF patient is eligible for treatment, no matter how rare their mutations.

References

Amatngalim, G. D., Rodenburg, L. W., Aalbers, B. L., Raeven, H. H., Aarts, E. M., Sarhane, D., Spelier, S., Lefferts, J. W., Silva, I. A., Nijenhuis, W., Vrendenbarg, S., Kruisselbrink, E., Brunsveld, J. E., Van Drunen, C. M., Michel, S., De Winter-De Groot, K. M., Heijerman, H. G., Kapitein, L. C., Amaral, M. D., . . . Beekman, J. M. (2022). Measuring cystic fibrosis drug responses in organoids derived from 2D differentiated nasal epithelia. *Life Science Alliance, 5(12)*, e202101320. https://doi.org/10.26508/lsa.202101320

Andersen, D. H. (1938). CYSTIC FIBROSIS OF THE PANCREAS AND ITS RELATION TO CELIAC DISEASE. *American Journal of Diseases of Children, 56(2)*, 344. https://doi.org/10.1001/archpedi.1938.01980140114013

Ashburn, T. T., & Thor, K. B. (2004). Drug repositioning: identifying and developing new uses for existing drugs. *Nature Reviews/Drug Discovery, 3(8)*, 673–683. https://doi.org/10.1038/nrd1468

Bartfeld, S., & Clevers, H. (2017). Stem cell-derived organoids and their application for medical research and patient treatment. *Journal of Molecular Medicine, 95(7),* 729–738. https://doi.org/10.1007/s00109-017-1531-7

Berkers, G., Van Mourik, P., Vonk, A. M., Kruisselbrink, E., Dekkers, J. F., De Winter-De Groot, K. M., Arets, H. G., Wilt, R. E. M. D., Dijkema, J. S., Vanderschuren, M. M., Houwen, R. H., Heijerman, H. G., Van De Graaf, E. A., Elias, S. G., Majoor, C. J., Koppelman, G. H., Roukema, J., Bakker, M., Janssens, H. M., . . . Van Der Ent, C. K. (2019). Rectal organoids enable personalized treatment of cystic fibrosis. *Cell Reports, 26(7)*, 1701-1708.e3. https://doi.org/10.1016/j.celrep.2019.01.068

Bose, S., Clevers, H., & Shen, X. (2021). Promises and challenges of organoid-guided precision medicine. *Med, 2(9)*, 1011–1026. https://doi.org/10.1016/j.medj.2021.08.005

Chakradhar, S. (2017). Put to the test: Organoid-based testing becomes a clinical tool. *Nature Medicine, 23(7)*, 796–799. https://doi.org/10.1038/nm0717-796

Corrò, C., Novellasdemunt, L., & Li, V. S. (2020). A brief history of organoids. *American Journal of Physiology. Cell Physiology, 319(1)*, C151–C165. https://doi.org/10.1152/ajpcell.00120.2020

Dankert-Roelse, J. E., Bouva, M. J., Jakobs, B. S., Janssens, H. M., De Winter-De Groot, K. M., Schönbeck, Y., Gille, J. J., Gulmans, V. A., Verschoof-Puite, R. K., Schielen, P. C., & Verkerk, P. H. (2019). Newborn blood spot screening for cystic fibrosis with a four-step screening strategy in the Netherlands. *Journal of Cystic Fibrosis, 18(1),* 54–63. https://doi.org/10.1016/j.jcf.2018.07.008

De Boeck, K., & Amaral, M. D. (2016). Progress in therapies for cystic fibrosis. *The Lancet. Respiratory Medicine, 4(8)*, 662–674. https://doi.org/10.1016/s2213-2600(16)00023-0

De Poel, E., Spelier, S., Hagemeijer, M., Van Mourik, P., Suen, S., Vonk, A., Brunsveld, J., Ithakisiou, G., Kruisselbrink, E., Oppelaar, H., Berkers, G., De Winter De Groot, K., Heida-Michel, S., Jans, S., Van Panhuis, H., Bakker, M., Van Der Meer, R., Roukema, J., Dompeling, E., . . . Beekman, J. (2023). FDA-approved drug screening in patient-derived organoids demonstrates potential of drug repurposing for rare cystic fibrosis genotypes. *Journal of Cystic Fibrosis, 22(3)*, 548–559. https://doi.org/10.1016/j.jcf.2023.03.004

De Winter-De Groot, K. M., Janssens, H. M., Van Uum, R. T., Dekkers, J. F., Berkers, G., Vonk, A., Kruisselbrink, E., Oppelaar, H., Vries, R., Clevers, H., Houwen, R. H., Escher, J. C., Elias, S. G., De Jonge, H. R., De Rijke, Y. B., Tiddens, H. A., Van Der Ent, C. K., & Beekman, J. M. (2018). Stratifying infants with cystic fibrosis for disease severity using intestinal organoid swelling as a biomarker of CFTR function. *European Respiratory Journal, 52(3)*, 1702529. https://doi.org/10.1183/13993003.02529-2017

Dekkers, J. F., Wiegerinck, C. L., De Jonge, H. R., Bronsveld, I., Janssens, H. M., De Winter-De Groot, K. M., Brandsma, A. M., De Jong, N. W. M., Bijvelds, M. J. C., Scholte, B. J., Nieuwenhuis, E. E. S., Van Den Brink, S., Clevers, H., Van Der Ent, C. K., Middendorp, S., & Beekman, J. M. (2013). A functional CFTR assay using primary cystic fibrosis intestinal organoids. *Nature Medicine, 19(7)*, 939–945. https://doi.org/10.1038/nm.3201

Dekkers, J. F., Berkers, G., Kruisselbrink, E., Vonk, A., De Jonge, H. R., Janssens, H. M., Bronsveld, I., Van De Graaf, E. A., Nieuwenhuis, E. E. S., Houwen, R. H. J., Vleggaar, F. P., Escher, J. C., De Rijke, Y. B., Majoor, C. J., Heijerman, H. G. M., De Winter–de Groot, K. M., Clevers, H., Van Der Ent, C. K., & Beekman, J. M. (2016). Characterizing responses to CFTR-modulating drugs using rectal organoids derived from subjects with cystic fibrosis. *Science Translational Medicine, 8(344)*. https://doi.org/10.1126/scitranslmed.aad8278

Eckford, P. D., Li, C., Ramjeesingh, M., & Bear, C. E. (2012). Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) potentiator VX-770 (IVACAFTOR) opens the defective channel gate of mutant CFTR in a phosphorylation-dependent but ATP-independent manner. *Journal of Biological Chemistry, 287(44)*, 36639–36649. https://doi.org/10.1074/jbc.m112.393637

Elborn, J. S. (2016). Cystic fibrosis. *Lancet, 388(10059)*, 2519–2531. https://doi.org/10.1016/s0140-6736(16)00576-6

Farrell, P. M., Rock, M. J., & Baker, M. W. (2020). The impact of the CFTR gene discovery on cystic fibrosis diagnosis, counseling, and preventive therapy. *Genes, 11(4)*, 401. https://doi.org/10.3390/genes11040401

Flume, P. A., Liou, T. G., Borowitz, D. S., Li, H., Yen, K., Ordoñez, C. L., & Geller, D. E. (2012). IVacAftor in subjects with cystic fibrosis who are homozygous for the F508DEL-CFTR mutation. *Chest, 142(3)*, 718–724. https://doi.org/10.1378/chest.11-2672

Gentzsch, M., & Mall, M. A. (2018). Ion channel modulators in cystic fibrosis. *Chest, 154(2), 383–393*. https://doi.org/10.1016/j.chest.2018.04.036

Gjorevski, N., Sachs, N., Manfrin, A., Giger, S., Bragina, M. E., Ordóñez-Morán, P., Clevers, H., & Lutolf, M. P. (2016). Designer matrices for intestinal stem cell and organoid culture. *Nature, 539(7630)*, 560–564. https://doi.org/10.1038/nature20168

Guo, J., Garratt, A., & Hill, A. (2022). Worldwide rates of diagnosis and effective treatment for

cystic fibrosis. *Journal of Cystic Fibrosis, 21(3)*, 456–462. https://doi.org/10.1016/j.jcf.2022.01.009

Hennig, A., Wolf, L., Jahnke, B., Polster, H., Seidlitz, T., Werner, K., Aust, D. E., Hampe, J., Distler, M., Weitz, J., Stange, D. E., & Welsch, T. (2019). CFTR Expression Analysis for subtyping of human Pancreatic cancer organoids. *Stem Cells International, 2019*, 1–8. https://doi.org/10.1155/2019/1024614

Kleinman, H. K., McGarvey, M. L., Hassell, J. R., Star, V. L., Cannon, F. B., Laurie, G. W., & Martin, G. R. (1986). Basement membrane complexes with biological activity. *Biochemistry, 25(2)*, 312–318. https://doi.org/10.1021/bi00350a005

Lancaster, M. A., & Knoblich, J. A. (2014). Organogenesis in a dish: Modeling development and disease using organoid technologies. *Science, 345(6194)*. https://doi.org/10.1126/science.1247125

López-Valdez, J. A., Aguilar-Alonso, L. A., Gándara-Quezada, V., Ruiz-Rico, G. E., Ávila-Soledad, J. M., Reyes, A. A., & Pedroza-Jiménez, F. D. (2021). Cystic fibrosis: current concepts. *Boletín Médico Del Hospital Infantil De México, 78(6)*. https://doi.org/10.24875/bmhim.20000372

Mall, M. A., & Hartl, D. (2014). CFTR: cystic fibrosis and beyond. *European Respiratory Journal, 44(4)*, 1042–1054. https://doi.org/10.1183/09031936.00228013

Munck, A., Kerem, E., Ellemunter, H., Campbell, D., Wang, L. T., Ahluwalia, N., Owen, C. A., & Wainwright, C. (2020). Tezacaftor/ivacaftor in people with cystic fibrosis heterozygous for minimal function CFTR mutations. *Journal of Cystic Fibrosis, 19(6)*, 962–968. https://doi.org/10.1016/j.jcf.2020.04.015

Ogawa, M., Jiang, J., Xia, S., Yang, D., Ding, A., Laselva, O., Hernandez, M., Cui, C., Higuchi, Y., Suemizu, H., Dorrell, C., Grompe, M., Bear, C. E., & Ogawa, S. (2021). Generation of functional ciliated cholangiocytes from human pluripotent stem cells. *Nature Communications, 12(1)*. https://doi.org/10.1038/s41467-021-26764-0

Okiyoneda, T., Veit, G., Dekkers, J. F., Bagdany, M., Soya, N., Xu, H., Roldan, A., Verkman, A. S., Kurth, M., Simon, A., Hegedus, T., Beekman, J. M., & Lukacs, G. L. (2013). Mechanism-based corrector combination restores ΔF508-CFTR folding and function. *Nature Chemical Biology, 9(7)*, 444–454. https://doi.org/10.1038/nchembio.1253

Ramalho, A. S., Amato, F., & Gentzsch, M. (2023). Patient-derived cell models for personalized medicine approaches in cystic fibrosis. *Journal of Cystic Fibrosis, 22,* S32–S38. https://doi.org/10.1016/j.jcf.2022.11.007

Regard, L., Martin, C., Da Silva, J., & Burgel, P. (2022). CFTR Modulators: Current status and Evolving knowledge. *Seminars in Respiratory and Critical Care Medicine, 44(02)*, 186–195. https://doi.org/10.1055/s-0042-1758851

Riordan, J. R., Rommens, J. M., Kerem, B., Alon, N., Rozmahel, R., Grzelczak, Z., Zielenski, J., Lok, S., Plavsic, N., Chou, J., Drumm, M. L., Iannuzzi, M. C., Collins, F. S., & Tsui, L. (1989). Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. *Science, 245(4922)*, 1066–1073. https://doi.org/10.1126/science.2475911

Sato, T., Vries, R. G., Snippert, H. J., Van De Wetering, M., Barker, N., Stange, D. E., Van Es, J.

H., Abo, A., Kujala, P., Peters, P. J., & Clevers, H. (2009). Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. *Nature, 459(7244),* 262–265. https://doi.org/10.1038/nature07935

Sato, T., Stange, D. E., Ferrante, M., Vries, R. G., Van Es, J. H., Van Den Brink, S., Van Houdt, W. J., Pronk, A., Van Gorp, J., Siersema, P. D., & Clevers, H. (2011). Long-term expansion of epithelial organoids from human colon, adenoma, adenocarcinoma, and Barrett's epithelium. *Gastroenterology, 141(5)*, 1762–1772. https://doi.org/10.1053/j.gastro.2011.07.050

Scotet, V., L'Hostis, C., & Férec, C. (2020). The Changing Epidemiology of Cystic Fibrosis: Incidence, survival and impact of the CFTR gene discovery. *Genes, 11(6)*, 589. https://doi.org/10.3390/genes11060589

Takahashi, K., Tanabe, K., Ohnuki, M., Narita, M., Ichisaka, T., Tomoda, K., & Yamanaka, S. (2007). Induction of Pluripotent Stem Cells from Adult Human Fibroblasts by Defined Factors. *Cell, 131(5)*, 861–872. https://doi.org/10.1016/j.cell.2007.11.019

The Clinical and Functional TRanslation of CFTR (CFTR2); CFTR2 Variant List History April 2023; available at http://cftr2.org

Van Mourik, P., Michel, S., Vonk, A. M., Beekman, J. M., & Van Der Ent, C. K. (2020). Rationale and design of the HIT-CF organoid study: stratifying cystic fibrosis patients based on intestinal organoid response to different CFTR-modulators. *Translational Medicine Communications, 5(1)*. https://doi.org/10.1186/s41231-020-00060-3

Wong, A. P., Bear, C. E., Chin, S., Pasceri, P., Thompson, T. O., Huan, L., Ratjen, F., Ellis, J., & Rossant, J. (2012). Directed differentiation of human pluripotent stem cells into mature airway epithelia expressing functional CFTR protein. *Nature Biotechnology, 30(9)*, 876–882. https://doi.org/10.1038/nbt.2328