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Current Opinion in Pharmacology

Established and novel human translational models to advance cystic fibrosis research, drug discovery, and optimize CFTR-targeting therapeutics

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Abstract

To find a cure for cystic fibrosis, there has been tremendous progress in the development of treatments that target the basic defect in the protein channel, CFTR. However, 10% of cystic fibrosis patients have rare CFTR mutations that are still without an approved CFTR-targeting drug. To identify relevant therapies for these patients, culture models using nasal, bronchial, and rectal tissue from individual patients allow functional, biochemical, and cellular detection of drug-rescued CFTR. Additionally, novel systems such as induced pluripotent stem cell-derived models are utilized to characterize CFTR mutations and identify treatments. State-of-the-art translational models were instrumental for CFTR modulator development and may become important for gene-based drug discovery and other novel therapeutic strategies.

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Current Opinion in Pharmacology 2022, **64**:102210

This review comes from a themed issue on **Respiratory**

Edited by **Carla M.P. Ribeiro** and **Martina Gentzsch**

For a complete overview see the Issue and the Editorial

Available online xxx

https://doi.org/10.1016/j.coph.2022.102210

Introduction

Cystic fibrosis (CF) is an autosomal recessive disease resulting from mutations in the CF transmembrane conductance regulator (CFTR) gene [1], which encodes an ion channel that transports chloride and bicarbonate, playing an important role in hydration of many epithelial surfaces. After discovery of the CFTR gene in 1989, preclinical research with in vitro human cell models paved the way for the development of CFTR-targeting therapeutics that permit successful treatment of the basic defect in CF. These CFTR modulators are small-molecular compounds known as correctors that augment transfer of mutant CFTR to the apical membrane, and potentiators that increase CFTR channel activity [2].

90% of people with CF (pwCF) in North America, and 80% worldwide carry the F508del CFTR mutation. Unfortunately, 10% of the CF population or more, depending on ethnicity, have rare mutations for which CFTR modulator therapies are not available. To reach these patients and to improve upon available therapies, numerous CFTR-targeting compounds and reagents are currently in the clinical pipeline including novel mRNA- and DNA-based gene therapy therapeutics, read-through reagents for premature stop codons, and advanced modulator compounds (https://www.cff.org/Trials/Pipeline), and thus, personalized models for CF research remain in high demand for predicting drug efficacy for CF individuals.

CF research has developed and employed specific, physiologically relevant human assay systems to advance discovery of CF drugs. Primary human bronchial epithelial (HBE) and nasal epithelial (HNE) cells are typically grown at air–liquid interface (ALI) as planar cultures to study electrophysiological properties that reflect the function of epithelial ion channels such as CFTR [3–7]. Such models are imperative for efficiently identifying and screening compounds before they enter clinical trials, maximizing the likelihood of achieving clinically meaningful improvements in CFTR function, thus facilitating rapid progression of clinical trials toward more effective CF treatments. This review will highlight new and improved models that have been utilized for identifying effective CFTR-targeting therapies for pwCF.

CF therapeutics

The first FDA-approved CFTR modulator was the potentiator ivacaftor (IVA), which is the active ingredient of a drug that improves the function of the CFTR gating mutant G551D [8,9]. While IVA or the CFTR

corrector lumacaftor (LUM) alone did not significantly improve lung function in the CFTR folding mutant, F508del [10], combining LUM with IVA or combining the newer corrector tezacaftor (TEZ) with IVA resulted in modest lung function improvements in clinical trials in pwCF homozygous for F508del CFTR [11–14]. The recently approved triple therapy drug (next-generation corrector elexacaftor (ELX) with TEZ and IVA; ELX/TEZ/IVA) showed substantial efficacy in phase 3 clinical trials [15,16] and was more robust than IVA and dual combination therapies in some populations. The improvement in lung function measured as forced expiratory volume in 1 s percent predicted compared with sex- and age-matched healthy lungs (FEV1pp) in pwCF receiving ELX/TEZ/IVA is significant, with an overall improvement of FEV1pp of at least 10% [16,17], which is comparable with what was observed in G551D pwCF treated with IVA. In addition, improvement in the gastrointestinal (GI) system was observed in patients taking ELX/TEZ/IVA.

Personalized models

The FDA approval of CFTR modulators for certain CFTR mutations was based on FRT cell line data. Although cell lines are useful to study mechanistic defects of CFTR mutations, they may not constitute a reliable physiological system for predicting all drug effects that are observed in epithelial tissues [18–21]. Some CFTR therapeutics such as read-through reagents prevented premature termination mutations in cell lines but did not work in primary epithelia where extensive nonsense-mediated mRNA decay is observed [22–25]. Identifying therapeutics that rescue CFTR splicing mutations are important for pwCF with such mutations. Studies on splicing mutations were previously conducted in cell lines but are more complicated in vivo and are therefore being intensively pursued [26]. In differentiated epithelia, CFTR localization and function are determined by cell type-specific expression and spatial interactions [27] and highly modulated by environmental factors such as inflammation [28].

Human in vitro models have been crucial for testing CFTR rescue in a relevant physiological environment. As patients respond differently to drugs, the most effective models for predicting clinical responses to therapeutics are cultures derived from tissue samples of individual patients; a personalized medicine approach. These cultures can be used in multiple assays to identify, optimize, and confirm therapies. In addition, it is important to have models available for examination of pharmacokinetics and pharmacodynamics of drugs [29].

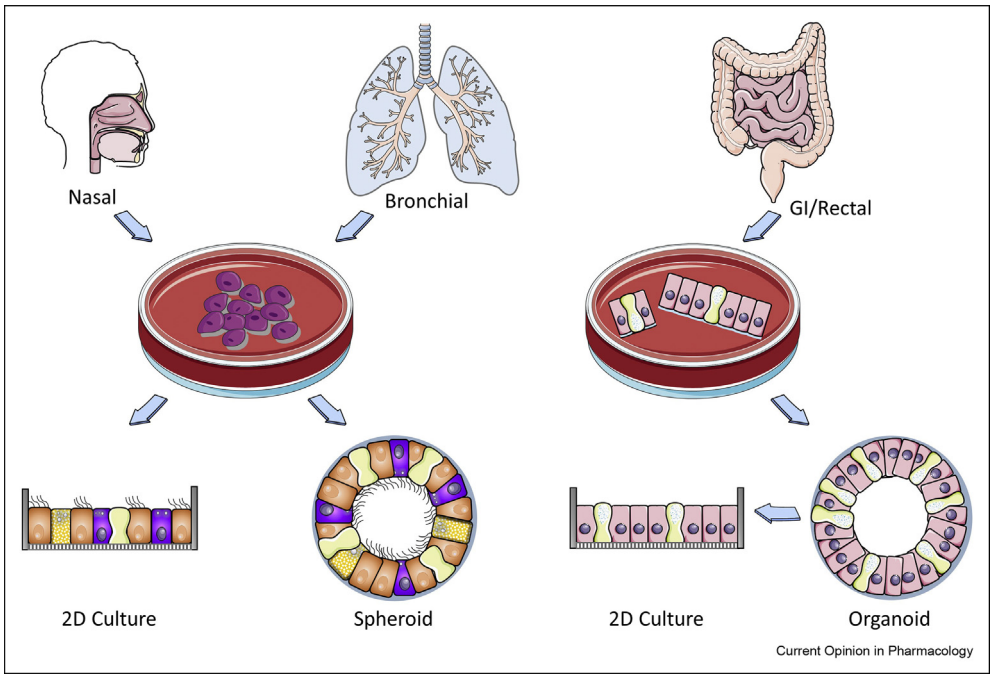
Well-established in vitro models are differentiated cultures of HBE and HNE cells on membranes at ALI [30]. ALI culture protocol details (i.e., passage number of cells, differentiation time, type of media, supplements, and insert type) may affect cell-type composition, the magnitude of ion channel expression and activity, and the amount of CFTR protein that is rescued [31,32]. A common method to assess mutant CFTR rescue in these cultures is to observe electrophysiological responses of CFTR measured as short-circuit currents in Ussing chambers by treatment with forskolin that leads to CFTR activation by cAMP-dependent protein kinase, PKA. Residual CFTR currents before further activation by forskolin may also be indicative of restored CFTR function, as well as a diminution of the epithelial sodium channel (ENaC) activity. Furthermore, activation of CFTR by nucleotides (i.e., UTP or ATP) in the presence of the calcium-activated chloride channel (CaCC) TMEM16A inhibitor offers the possibility to evaluate an additional physiological activation route. Restoration of mucociliary clearance (MCC) of CF HNE and HBE ALI cultures is less commonly used for primary drug screenings; however, they can be investigated to evaluate the ability of a CFTR-targeting drug to restore airway surface liquid homeostasis, normalize mucin concentrations, and ciliary dynamics [33,34], which are all important components of a well-functioning MCC. This demonstrates the importance of CFTR modulators on airway hydration and mucus properties [33].

Patient specimens from airway and GI tissue can be used to generate organoids for testing rescue of mutant CFTR function [35–37]. Depending on the culture method, airway organoids can be oriented such that the apical membrane faces inward or outward, and rescue of mutant CFTR can be quantitated by changes in organoid size. These methods vary by tissue type and are described in more detail, below. An overview of the most commonly used models to evaluate CFTR therapeutics is shown in Figure 1.

Bronchial cultures

Primary HBE cells derived from explant lungs of pwCF and cultured at ALI have been the gold standard for studying the efficacy of CFTR modulators [8,38,39]. In addition, HBE can be obtained from living patients via bronchial brushings, which provide the potential to identify optimal therapeutics for individual patients (personalized medicine). To expand resources available for testing of CFTR modulators, CF HBE cells can be conditionally reprogrammed and maintained to higher passage numbers [32]. Organoids/spheroids from airway epithelial cells are obtained by seeding of CF HBE in matrigel, in which the movement of ions by luminal CFTR channels drives fluid toward the center, resulting in swelling that is not observed in CF patient-derived organoids where CFTR is defective. These models are suitable to quantitate CFTR-targeting therapeutics that induce CFTR-dependent spheroid swelling and are also an appropriate model to study CF pathophysiology [37,40]. In addition, a novel method for generating

Figure 1



Translational human in vitro models for CF research and drug discovery. Processing and expansion of nasal, bronchial, and GI epithelial tissues to form 2D planar and 3D spheroid cultures. Expanded HNE and HBE cells are either seeded on membranes form planar cultures or in matrigel to form spheroids. GI organoids develop directly from partially digested tissues. Planar and spheroid cultures can be utilized to evaluate pharmacological rescue of CFTR by various assays that evaluate CFTR function and maturation. Some image panels were obtained from Servier Medical Art (smart.servier.com).

bronchial organoids with externally oriented apical membranes in mixed matrix components was recently published [41]. Expanded cultures of bronchial epithelial cells can be utilized as an additional source of tissue to create spheroids for more testing of CFTR-targeting therapeutics.

Nasal cultures

HNE cells have many properties in common with HBE cells and form polarized, pseudostratified epithelia mimicking in vivo airways and the expression of ion channels including CFTR, ENaC, and CaCCs. Similar to HBE cells, HNE cells can be derived by brushing or scraping but the collection is far less invasive than for HBE cells [42]. Thus, patient-derived HNE cultures differentiated at ALI have become a standard model in CFTR modulator testing [42–49]. Epithelial cell types such as ionocytes, ciliated cells, and secretory cells differ between nasal cells and large (bronchi) and small (bronchioles) airways [50–53]; however, HNE cultures appear to recapitulate many of the bioelectric properties of differentiated HBE and respond in a similar fashion to CFTR modulators [42,44,54]. HBE and HNE cultures can be used to evaluate the effects of CFTR modulators on reversing secondary CF phenotypes such as decreased MCC. Similar to HBE, conditionally reprogramming of HNE is available as a method to expand patient culture lifetime without majorly affecting CFTR function [55,56]. CFTR-mediated chloride currents in HNE cells correlated with patients' sweat chloride concentrations, a common method to detect CF [55]. Thus, HNE cells are recognized as a non-invasive surrogate for HBE cells in many preclinical studies of CFTR modulators [36,42–46,55,57–63]. Patient-derived nasal tissue can be grown in suspension, creating nasospheroids with CFTR channels on the outer surface [36]. Upon rescue of mutant CFTR, ion transport and fluid will move outward toward the media, causing the spheroids to shrink, which can be quantitated [36].

Intestinal cultures

In 2013, it was shown that organoids derived from patients' intestines can be utilized to study drug rescue of mutant CFTR function [64]. A recent study showed that intestinal organoids can be used to test for CFTR rescue of nonsense mutations, in which 5 different therapies were combined: 3 CFTR modulators (ELX/TEZ/IVA) a compound that induces translational read through, and a compound that inhibits nonsense mRNA-mediated decay. This is very promising for pwCF with nonsense CFTR mutations that are not eligible for the triple therapy, ELX/TEZ/IVA [65]. Another recent

study using intestinal organoids showed the importance of testing compounds from different companies to optimize efficacy of F508del CFTR rescue [66]. Although F508del CFTR in patient-derived cultures typically responds well to rescue by triple therapy, cultures from different patients with the same CFTR genotype do not respond in a similar fashion, which may be due to genetic traits other than CFTR that may affect CFTR modulator efficacy and therefore should be further examined [67,68]. Additional models using different intestinal tissues and different culture methods/scaffolds may also have the potential to be used for CF research [69–71].

As clinical improvement of pwCF and in vitro readout by rectal organoids appear to be correlated [65,72,73], large efforts such as HIT-CF Europe aim to expand organoid-based screenings of drugs from multiple companies to pwCF with rare mutations (https://www.hitcf.org/). Furthermore, GI organoids are not only utilized for selection of optimized treatments for rare CFTR mutations but also for evaluation of novel read-through therapies for nonsense mutations and gene therapeutic approaches [74,75]. Additionally, GI organoids can be seeded on membranes to form monolayers that develop into differentiated cultures that are analyzed electrophysiologically in Ussing chambers to study restoration of CFTR-mediated currents in CF planar cultures [76]. Recent publications demonstrated the power of directly comparing clinical data with in vitro data from patient-derived intestinal cultures [77,78]. GI organoids are the most straightforward model to develop from only partially digested tissue specimens. These can be subsequently cultured on inserts as monolayers, offering the following advantages: 1) they can be analyzed in Ussing chambers, and 2) the apical surface is directly accessible for treatments.

Other gastrointestinal cultures

CF patients frequently suffer from CF-related diabetes (CFRD), which can lead to glucose imbalance that can in turn, augment the severity of CF disease. It is therefore important to develop a relevant pancreas model for testing potential therapies that improve these glucose imbalances. A pancreas-on-a-chip model was developed using patient-derived pancreatic ductal epithelial cells (PDECs) and pancreatic islets, allowing for examination of the relationship between these cell types [79]. CFTR is expressed in PDECs and inhibition of CFTR channel function leads to a decrease in insulin secretion. To alleviate the effects of CFRD, this pancreas-on-a-chip model can be utilized to test CFTR modulators for their ability to improve glucose imbalances in individual patients. In addition, patient-derived cultures of biliary tissue (cholangiocytes) can also be used to test for rescue of CFTR function [80,81].

Induced pluripotent stem cells

Induced pluripotent stem cells (iPSCs) are adult somatic (e.g., skin or blood) cells that have been reprogrammed, bringing the cells back to an embryonic-like pluripotent state [82]. This allows the creation of an unlimited source of any type of human cell for therapeutic testing. Although creating lung tissue from stem cells is very complicated, in 2015, iPSCs were created from F508del pwCF, corrected with wild-type CFTR gene sequences, and then differentiated into airway epithelial cells [83]. iPSC-derived lung epithelium can be used to generate lung organoids that mimic lung tissue [84–86]. Furthermore, iPSCs can be used to model defects in ciliary function, which can be beneficial for measuring defective MCC in CF cultures [87]. iPSC-derived lung progenitor cells can be set up in a high-throughput platform, allowing studies that measure rescue of mutant CFTR function [88]. In addition, for GI studies, iPSCs were used to create pancreatic duct-like organoids that expressed CFTR [89]. Overall, iPSC technology may be particularly beneficial for pwCF with rare CFTR mutations such as nonsense mutations that do not yet have approved CFTR-targeting therapies.

Preclinical models in development

Exciting developments are ongoing using cells from other tissues such as sweat ducts, submucosal glands, and liver [90–92] for translation modeling of CF. As CF disease is thought to be initiated in small airways, HBE cells from small and large airways are utilized as separate models to display typical characteristics of cell populations found in these specific regions [50]. To study engraftment with HBE cultures expressing wild-type CFTR, a cell therapy approach was developed involving repopulation CF HBE cultures, thereby creating a population of cells that express CFTR with normal function [93]. Advanced models aim to incorporate environmental conditions found in the CF lungs such as infection, inflammation, mucus burden, and hypoxia [28,94]. In testing various drugs for their ability to repair the defects of CFTR mutations, it is important to consider how the efficacy of drugs may be affected by nearby tissues, cells, and secreted reagents such as the endothelium, immune system cells and cytokines, and bacteria. To address this, a CF Airway Chip was created that includes CF bronchial epithelial cells grown at air–liquid interface, lung endothelium, and dynamic fluid flow that can deliver immune cells [95]. These CF Airway Chips accurately represent the human airway in vivo, allowing direct testing of various drugs to rescue rare CFTR mutations. Other organs-on-a-chip are also in development for CF research, including multi-tissue organs-on-a-chip to address the effects of different organs on each other [96,97].

Conclusions

In vitro models derived from tissues of pwCF are vital for the development of effective CFTR therapeutics. Personalized models are important as patients respond differently to treatments. Using tissue that is relevant to CF (bronchial, nasal, and GI epithelial cells) collected from pwCF for drug testing is an effective method for identifying and optimizing therapeutics for each patient. Cultures grown in planar (2D) and spheroid or organoid (3D) formats are used as models of CF disease in multiple drug testing assays. These in vitro translational models have been crucial for understanding CF pathophysiology and CFTR regulation and were instrumental in identifying effective CFTR modulators for pwCF. Newer models such as iPSC-derived cultures and organs-on-a-chip allow the use of additional material and more advanced methods, respectively. In the future, researchers will continue to tackle remaining complicated cellular issues with models that simulate inflammation, infection, mucus, and MCC, enabling gene therapy studies that accurately predict clinical outcomes for pwCF in need of effective therapies.

Author contributions

D.M.C. and M.G. conceptualized and wrote the review.

Conflict of interest statement

Nothing declared.

Acknowledgements

We thank members of the Marsico Lung Institute, UNC Cystic Fibrosis Research and Translation Core Center and Research and Development Program Cores for their contributions to translation research discussed here. Related research was supported by the Cystic Fibrosis Foundation (GENTZS18P0, GENTZS19I0, BOUCHE19R0) and the National Institutes of Health (P30DK065988).

References

Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest
** of outstanding interest

1. Riordan JR, Rommens JM, Kerem B, Alon N, Rozmahel R, Grzelczak Z, Zielenski J, Lok S, Plavsic N, Chou JL, *et al.*: **Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA**. *Science* 1989, **245**: 1066–1073.

2. Gentzsch M, Mall MA: **Ion channel modulators in cystic fibrosis**. *Chest* 2018, **154**:383–393.

3. Randell SH, Fulcher ML, O'Neal W, Olsen JC: **Primary epithelial cell models for cystic fibrosis research**. *Methods Mol Biol* 2011, **742**:285–310.

4. Muller L, Brighton LE, Carson JL, Fischer 2nd WA, Jaspers I: **Culturing of human nasal epithelial cells at the air liquid interface**. *JoVE* 2013, **80**:e50646.

5. de Courcey F, Zholos AV, Atherton-Watson H, Williams MT, Canning P, Danahay HL, Elborn JS, Ennis M: **Development of primary human nasal epithelial cell cultures for the study of cystic fibrosis pathophysiology**. *Am J Physiol Cell Physiol* 2012, **303**:C1173–C1179.

6. Wiszniewski L, Jornot L, Dudez T, Pagano A, Rochat T, Lacroix JS, Suter S, Chanson M: **Long-term cultures of polarized airway epithelial cells from patients with cystic fibrosis**. *Am J Respir Cell Mol Biol* 2006, **34**:39–48.

7. Mosler K, Coraux C, Fragaki K, Zahm JM, Bajolet O, Bessaci-Kabouya K, Puchelle E, Abely M, Mauran P: **Feasibility of nasal epithelial brushing for the study of airway epithelial functions in CF infants**. *J Cyst Fibros* 2008, **7**:44–53.

8. Van Goor F, Hadida S, Grootenhuis PD, Burton B, Cao D, Neuberger T, Turnbull A, Singh A, Joubran J, Hazlewood A, *et al.*: **Rescue of CF airway epithelial cell function in vitro by a CFTR potentiator, VX-770**. *Proc Natl Acad Sci U S A* 2009, **106**: 18825–18830.

9. Ramsey BW, Davies J, McElvaney NG, Tullis E, Bell SC, Drevinek P, Griese M, McKone EF, Wainwright CE, Konstan MW, *et al.*: **A CFTR potentiator in patients with cystic fibrosis and the G551D mutation**. *N Engl J Med* 2011, **365**:1663–1672.

10. Clancy JP, Rowe SM, Accurso FJ, Aitken ML, Amin RS, Ashlock MA, Ballmann M, Boyle MP, Bronsveld I, Campbell PW, *et al.*: **Results of a phase IIa study of VX-809, an investigational CFTR corrector compound, in subjects with cystic fibrosis homozygous for the F508del-CFTR mutation**. *Thorax* 2012, **67**:12–18.

11. Boyle MP, Bell SC, Konstan MW, McColley SA, Rowe SM, Rietschel E, Huang X, Waltz D, Patel NR, Rodman D: **A CFTR corrector (lumacaftor) and a CFTR potentiator (ivacaftor) for treatment of patients with cystic fibrosis who have a phe508del CFTR mutation: a phase 2 randomised controlled trial**. *Lancet Respir Med* 2014, **2**:527–538.

12. Wainwright CE, Elborn JS, Ramsey BW, Marigowda G, Huang X, Cipolli M, Colombo C, Davies JC, De Boeck K, Flume PA, *et al.*: **Lumacaftor-Ivacaftor in Patients with Cystic Fibrosis Homozygous for Phe508del CFTR**. *N Engl J Med* 2015, **373**:220–231.

13. Donaldson SH, Pilewski JM, Griese M, Cooke J, Viswanathan L, Tullis E, Davies JC, Lekstrom-Himes JA, Wang LT: **Tezacaftor/Ivacaftor in Subjects with Cystic Fibrosis and F508del/F508del-CFTR or F508del/G551D-CFTR**. *Am J Respir Crit Care Med* 2018, **197**:214–224.

14. Taylor-Cousar JL, Munck A, McKone EF, van der Ent CK, Moeller A, Simard C, Wang LT, Ingenito EP, McKee C, Lu Y, *et al.*: **Tezacaftor-Ivacaftor in Patients with Cystic Fibrosis Homozygous for Phe508del**. *N Engl J Med* 2017, **377**: 2013–2023.

15. Heijerman HGM, McKone EF, Downey DG, Van Braeckel E, Rowe SM, Tullis E, Mall MA, Welter JJ, Ramsey BW, McKee CM, *et al.*: **Efficacy and safety of the elexacaftor plus tezacaftor plus ivacaftor combination regimen in people with cystic fibrosis homozygous for the F508del mutation: a double-blind, randomised, phase 3 trial**. *Lancet* 2019, **394**:1940–1948.

16. Middleton PG, Mall MA, Drevinek P, Lands LC, McKone EF, Polineni D, Ramsey BW, Taylor-Cousar JL, Tullis E, Vermeulen F, *et al.*: **Elexacaftor-Tezacaftor-Ivacaftor for Cystic Fibrosis with a Single Phe508del Allele**. *N Engl J Med* 2019, **381**: 1809–1819.

17. Zemanick ET, Taylor-Cousar JL, Davies J, Gibson RL, Mall MA, McKone EF, McNally P, Ramsey BW, Rayment JH, Rowe SM, *et al.*: **A Phase 3 Open-Label Study of Elexacaftor/Tezacaftor/Ivacaftor in Children 6 through 11 Years of Age with Cystic Fibrosis and at Least One F508del Allele**. *Am J Respir Crit Care Med* 2021, **203**:1522–1532.

18. Pedemonte N, Tomati V, Sondo E, Galietta LJ: **Influence of cell background on pharmacological rescue of mutant CFTR**. *Am J Physiol Cell Physiol* 2010, **298**:C866–C874.

19. Rowe SM, Pyle LC, Jurkevante A, Varga K, Collawn J, Sloane PA, Woodworth B, Mazur M, Fulton J, Fan L, *et al.*: **DeltaF508 CFTR processing correction and activity in polarized airway and non-airway cell monolayers**. *Pulm Pharmacol Therapeut* 2010, **23**:268–278.

20. Ostedgaard LS, Rogers CS, Dong Q, Randak CO, Vermeer DW, Rokhlina T, Karp PH, Welsh MJ: **Processing and function of**

CFTR-DeltaF508 are species-dependent. *Proc Natl Acad Sci U S A* 2007, **104**:15370–15375.

21. Bebok Z, Collawn JF, Wakefield J, Parker W, Li Y, Varga K, Sorscher EJ, Clancy JP: **Failure of cAMP agonists to activate rescued deltaF508 CFTR in CFBE41o- airway epithelial monolayers**. *J Physiol* 2005, **569**:601–615.

22. Haggie PM, Phuan PW, Tan JA, Xu H, Avramescu RG, Perdomo D, Zlock L, Nielson DW, Finkbeiner WE, Lukacs GL, *et al.*: **Correctors and potentiators rescue function of the truncated W1282X-cystic fibrosis transmembrane regulator (CFTR) translation product**. *J Biol Chem* 2017, **292**:771–785.

23. Mutyam V, Libby EF, Peng N, Hadjiliadis D, Bonk M, Solomon GM, Rowe SM: **Therapeutic benefit observed with the CFTR potentiator, ivacaftor, in a CF patient homozygous for the W1282X CFTR nonsense mutation**. *J Cyst Fibros* 2017, **16**: 24–29.

24. Hamosh A, Rosenstein BJ, Cutting GR: **CFTR nonsense mutations G542X and W1282X associated with severe reduction of CFTR mRNA in nasal epithelial cells**. *Hum Mol Genet* 1992, **1**: 542–544.

25. Aksit MA, Bowling AD, Evans TA, Joynt AT, Osorio D, Patel S, West N, Merlo C, Sosnay PR, Cutting GR, *et al.*: **Decreased mRNA and protein stability of W1282X limits response to modulator therapy**. *J Cyst Fibros* 2019, **18**:606–613.

26. Dang Y, van Heusden C, Nickerson V, Chung F, Wang Y, Quinney NL, Gentzsch M, Randell SH, Moulton HM, Kole R, *et al.*: **Enhanced delivery of peptide-morpholino oligonucleotides with a small molecule to correct splicing defects in the lung**. *Nucleic Acids Res* 2021, **49**:6100–6113.

27. Gentzsch M, Farinha CM: **Revisiting CFTR interactions: old partners and new players**. *Int J Mol Sci* 2021, **22**:13196.

28. * Ribeiro CMP, Gentzsch M: **Impact of airway inflammation on the efficacy of CFTR modulators**. *Cells* 2021, **10**:3260.
This paper is a review but also provides new data showing that CFTR rescue by modulators in primary human bronchial epithelial cultures is increased by airway inflammation, which is important to consider for drug testing.

29. Guhr Lee TN, Cholon DM, Quinney NL, Gentzsch M, Esther Jr CR: **Accumulation and persistence of ivacaftor in airway epithelia with prolonged treatment**. *J Cyst Fibros* 2020, **19**:746–751.

30. Fulcher ML, Randell SH: **Human nasal and tracheo-bronchial respiratory epithelial cell culture**. *Methods Mol Biol* 2013, **945**: 109–121.

31. Rayner RE, Makena P, Prasad GL, Cormet-Boyaka E: **Optimization of normal human bronchial epithelial (NHBE) cell 3D cultures for in vitro lung model studies**. *Sci Rep* 2019, **9**:500.

32. Gentzsch M, Boyles SE, Cheluvaraju C, Chaudhry IG, Quinney NL, Cho C, Dang H, Liu X, Schlegel R, Randell SH: **Pharmacological rescue of conditionally reprogrammed cystic fibrosis bronchial epithelial cells**. *Am J Respir Cell Mol Biol* 2017, **56**:568–574.

33. Morrison CB, Shaffer KM, Araba KC, Markovetz MR, Wykoff JA, Quinney NL, Hao S, Delion MF, Flen AL, Morton LC, *et al.*: **Treatment of cystic fibrosis airway cells with CFTR modulators reverses aberrant mucus properties via hydration**. *Eur Respir J* 2022, **59**:2100185.

34. Chioccioli M, Feriani L, Kotar J, Bratcher PE, Cicuta P: **Phenotyping ciliary dynamics and coordination in response to CFTR-modulators in Cystic Fibrosis respiratory epithelial cells**. *Nat Commun* 2019, **10**:1763.

35. Boj SF, Vonk AM, Statia M, Su J, Vries RR, Beekman JM, Clevers H: **Forskolin-induced swelling in intestinal organoids: an in vitro assay for assessing drug response in cystic fibrosis patients**. *JoVE* 2017, **120**:e55159.

36. Guimbellot JS, Leach JM, Chaudhry IG, Quinney NL, Boyles SE, Chua M, Aban I, Jaspers I, Gentzsch M: **Nasospheroids permit measurements of CFTR-dependent fluid transport**. *JCI Insight* 2017, **2**:e95734.

37. Sachs N, Papaspyropoulos A, Zomer-van Ommen DD, Heo I, Bottinger L, Klay D, Weeber F, Huelsz-Prince G, Iakobachvili N, Amatngalim GD, *et al.*: **Long-term expanding human airway organoids for disease modeling**. *EMBO J* 2019, **38**:e100300.

38. Van Goor F, Hadida S, Grootenhuis PD, Burton B, Stack JH, Straley KS, Decker CJ, Miller M, McCartney J, Olson ER, *et al.*: **Correction of the F508del-CFTR protein processing defect in vitro by the investigational drug VX-809**. *Proc Natl Acad Sci U S A* 2011, **108**:18843–18848.

39. Keating D, Marigowda G, Burr L, Daines C, Mall MA, McKone EF, Ramsey BW, Rowe SM, Sass LA, Tullis E, *et al.*: **VX-445-Tezacaftor-Ivacaftor in Patients with Cystic Fibrosis and One or Two Phe508del Alleles**. *N Engl J Med* 2018, **379**:1612–1620.

40. Cholon DM, Gentzsch M: **Recent progress in translational cystic fibrosis research using precision medicine strategies**. *J Cyst Fibros* 2018, **17**:S52–S60.

41. Boecking CA, Walentek P, Zlock LT, Sun DI, Wolters PJ, Ishikawa H, Jin BJ, Haggie PM, Marshall WF, Verkman AS, *et al.*: **A simple method to generate human airway epithelial organoids with externally-oriented apical membranes**. *Am J Physiol Lung Cell Mol Physiol* 2022, **322**:L420–L437.

42. ** Brewington JJ, Filbrandt ET, LaRosa 3rd FJ, Moncivaiz JD, Ostmann AJ, Strecker LM, Clancy JP: **Brushed nasal epithelial cells are a surrogate for bronchial epithelial CFTR studies**. *JCI Insight* 2018, **3**:e99385.
To test whether nasal epithelial cells can recapitulate in vitro drug responses seen in bronchilal cells, this study used nasal and bronchial samples from pwCF with various CFTR mutations to compare the 2 tissue types. The results showed very similar CFTR function in response to CFTR modulators between HNE and HBE. These data confirm that HNE cell cultures mimic CFTR characteristics seen in HBE and are therefore a suitable HBE surrogate for testing drug rescue of CFTR.

43. Pranke IM, Hatton A, Simonin J, Jais JP, Le Pimpec-Barthes F, Carsin A, Bonnette P, Fayon M, Stremler-Le Bel N, Grenet D, *et al.*: **Correction of CFTR function in nasal epithelial cells from cystic fibrosis patients predicts improvement of respiratory function by CFTR modulators**. *Sci Rep* 2017, **7**:7375.

44. Kmit A, Marson FAL, Pereira SV, Vinagre AM, Leite GS, Servidoni MF, Ribeiro JD, Ribeiro AF, Bertuzzo CS, Amaral MD: **Extent of rescue of F508del-CFTR function by VX-809 and VX-770 in human nasal epithelial cells correlates with SNP rs7512462 in SLC26A9 gene in F508del/F508del Cystic Fibrosis patients**. *Biochim Biophys Acta (BBA) - Mol Basis Dis* 2019, **1865**:1323–1331.

45. ** Clarke LA, Awatade NT, Felicio VM, Silva IA, Calucho M, Pereira L, Azevedo P, Cavaco J, Barreto C, Bertuzzo C, *et al.*: **The effect of premature termination codon mutations on CFTR mRNA abundance in human nasal epithelium and intestinal organoids: a basis for read-through therapies in cystic fibrosis**. *Hum Mutat* 2019, **40**:326–334.
This study used quantitative real time PCR to measure variations in CFTR mRNA levels for several premature termination codon (PTC) mutations in human nasal epithelia and intestinal organoids and found reduced amounts of mRNA transcripts compared to non-PTC mutations. These results demonstrate the relevance of using nasal and intestinal models in predicting efficacy of PTC read-through drugs.

46. Leir SH, Yin S, Kerschner JL, Xia S, Ahmadi S, Bear C, Harris A: **An organoid model to assay the role of CFTR in the human epididymis epithelium**. *Cell Tissue Res* 2020, **381**:327–336.

47. Laselva O, Moraes TJ, He G, Bartlett C, Szarics I, Ouyang H, Gunawardena TNA, Strug L, Bear CE, Gonska T: **The CFTR mutation c.3453G > C (D1152H) confers an anion selectivity defect in primary airway tissue that can Be rescued by ivacaftor**. *J Personalized Med* 2020, **10**:40.

48. Laselva O, Bartlett C, Popa A, Ouyang H, Gunawardena TNA, Gonska T, Moraes TJ, Bear CE: **Emerging preclinical modulators developed for F508del-CFTR have the potential to be effective for ORKAMBI resistant processing mutants**. *J Cyst Fibros* 2021, **20**:106–119.

49. Pedemonte N: **Nasal epithelial cells as a gold-standard predictive model for personalized medicine in cystic fibrosis**. *J Physiol* 2022, **600**:1285–1286.

50. Okuda K, Dang H, Kobayashi Y, Carraro G, Nakano S, Chen G, Kato T, Asakura T, Gilmore RC, Morton LC, *et al.*: **Secretory cells dominate airway CFTR expression and function in human airway superficial epithelia**. *Am J Respir Crit Care Med* 2021, **203**:1275–1289.

51. Scudieri P, Musante I, Venturini A, Guidone D, Genovese M, Cresta F, Caci E, Palleschi A, Poeta M, Santamaria F, *et al.*: **Ionocytes and CFTR chloride channel expression in normal and cystic fibrosis nasal and bronchial epithelial cells**. *Cells* 2020, **9**:2090.

52. Plasschaert LW, Zilionis R, Choo-Wing R, Savova V, Knehr J, Roma G, Klein AM, Jaffe AB: **A single-cell atlas of the airway epithelium reveals the CFTR-rich pulmonary ionocyte**. *Nature* 2018, **560**:377–381.

53. Montoro DT, Haber AL, Biton M, Vinarsky V, Lin B, Birket SE, Yuan F, Chen S, Leung HM, Villoria J, *et al.*: **A revised airway epithelial hierarchy includes CFTR-expressing ionocytes**. *Nature* 2018, **560**:319–324.

54. ** Anderson JD, Liu Z, Odom LV, Kersh L, Guimbellot JS: **CFTR function and clinical response to modulators parallel nasal epithelial organoid swelling**. *Am J Physiol Lung Cell Mol Physiol* 2021, **321**:L119–L129.

This study demonstrates correlations between nasal organoid swelling assays, short- circuit current measurements, and clinical data from individual CF patients, indicating that in vitro culture data can be used to predict drug responses in patients.

55. * Noel S, Servel N, Hatton A, Golec A, Rodrat M, Ng DRS, Li H, Pranke I, Hinzpeter A, Edelman A, *et al.*: **Correlating genotype with phenotype using CFTR-mediated whole-cell Cl(-) currents in human nasal epithelial cells**. *J Physiol* 2021, **600**: 1515–1531.

HNE were conditionally reprogrammed to extend the lifetime of patient cultures, which did not majorly affect CFTR function. In addition, CFTR-mediated chloride currents in HNE cells were compared with sweat chloride concentrations, and a direct correlation was found, indicating the relevance of conditionally reprogrammed HNE as a model for predicting clinical outcomes.

56. Sette G, Lo Cicero S, Blacona G, Pierandrei S, Bruno SM, Salvati V, Castelli G, Falchi M, Fabrizzi B, Cimino G, *et al.*: **Theratyping cystic fibrosis in vitro in ALI culture and organoid models generated from patient-derived nasal epithelial conditionally reprogrammed stem cells**. *Eur Respir J* 2021, **58**: 2100908.

57. McCarthy C, Brewington JJ, Harkness B, Clancy JP, Trapnell BC: **Personalised CFTR pharmacotherapeutic response testing and therapy of cystic fibrosis**. *Eur Respir J* 2018, **51**:1702457.

58. * Brewington JJ, Filbrandt ET, LaRosa 3rd FJ, Ostmann AJ, Strecker LM, Szczesniak RD, Clancy JP: **Detection of CFTR function and modulation in primary human nasal cell spheroids**. *J Cyst Fibros* 2018, **17**:26–33.

HNE from patients were expanded and grown as spheroids and used to test for rescue of F508del CFTR in response to available CFTR modulators. These results demonstrate that spheroid swelling assays conducted in patient-derived HNE are a good model to quantitate drug rescue of mutant CFTR.

59. Brewington JJ, Filbrandt ET, LaRosa 3rd FJ, Moncivaiz JD, Ostmann AJ, Strecker LM, Clancy JP: **Generation of human nasal epithelial cell spheroids for individualized cystic fibrosis transmembrane conductance regulator study**. *JoVE* 2018, **134**: 57492.

60. ** McCravy MS, Quinney NL, Cholon DM, Boyles SE, Jensen TJ, Aleksandrov AA, Donaldson SH, Noone PG, Gentzsch M: **Personalised medicine for non-classic cystic fibrosis resulting from rare CFTR mutations**. *Eur Respir J* 2020, **56**:2000062.

HNE cultures from a patient with non-classic CF were used to conduct a panel of assays to examine CFTR function, protein expression, and maturation in order determine effective drug therapies. This study demonstrates the power of precision medicine models to support diagnosis and treatment for all CF patients.

61. Gentzsch M, Ren HY, Houck SA, Quinney NL, Cholon DM, Sopha P, Chaudhry IG, Das J, Dokholyan NV, Randell SH, *et al.*: **Restoration of R117H CFTR folding and function in human airway cells through combination treatment with VX-809 and VX-770**. *Am J Physiol Lung Cell Mol Physiol* 2016, **311**: L550–L559.

62. Laselva O, McCormack J, Bartlett C, Ip W, Gunawardena TNA, Ouyang H, Eckford PDW, Gonska T, Moraes TJ, Bear CE: **Preclinical studies of a rare CF-causing mutation in the second nucleotide binding domain (c.3700A>G) show robust functional rescue in primary nasal cultures by novel CFTR modulators**. *J Personalized Med* 2020, **10**:209.

63. Laselva O, Bartlett C, Gunawardena TNA, Ouyang H, Eckford PDW, Moraes TJ, Bear CE, Gonska T: **Rescue of multiple class II CFTR mutations by elexacaftor+tezacaftor+-ivacaftor mediated in part by the dual activities of elexacaftor as both corrector and potentiator**. *Eur Respir J* 2021, **57**.

64. Dekkers JF, Wiegerinck CL, de Jonge HR, Bronsveld I, Janssens HM, de Winter-de Groot KM, Brandsma AM, de Jong NW, Bijvelds MJ, Scholte BJ, *et al.*: **A functional CFTR assay using primary cystic fibrosis intestinal organoids**. *Nat Med* 2013, **19**:939–945.

65. de Poel E, Spelier S, Suen SWF, Kruisselbrink E, Graeber SY, Mall MA, Weersink EJM, van der Eerden MM, Koppelman GH, van der Ent CK, *et al.*: **Functional restoration of CFTR nonsense mutations in intestinal organoids**. *J Cyst Fibros* 2021, **21**:246–253.

66. ** de Poel E, Spelier S, Korporaal R, Lai KW, Boj SF, Conrath K, van der Ent CK, Beekman JM: **CFTR rescue in intestinal organoids with GLPG/ABBV-2737, ABBV/GLPG-2222 and ABBV/GLPG-2451 triple therapy**. *Front Mol Biosci* 2021, **8**:698358.

This study showed that intestinal organoids treated with a combination of 5 different therapies allowed the detection of rescue of CFTR nonsense mutations. This is a very important strategy for discovering therapies for pwCF with rare CFTR mutations that are not eligible for ELX/TEZ/IVA.

67. Furstova E, Dousova T, Beranek J, Libik M, Fila L, Modrak M, Cinek O, Macek Jr M, Drevinek P: **Response to elexacaftor/tezacaftor/ivacaftor in intestinal organoids derived from people with cystic fibrosis**. *J Cyst Fibros* 2021, **21**:243–245.

68. Gong J, Wang F, Xiao B, Panjwani N, Lin F, Keenan K, Avolio J, Esmaeili M, Zhang L, He G, *et al.*: **Genetic association and transcriptome integration identify contributing genes and tissues at cystic fibrosis modifier loci**. *PLoS Genet* 2019, **15**, e1008007.

69. Wang Y, DiSalvo M, Gunasekara DB, Dutton J, Proctor A, Lebhar MS, Williamson IA, Speer J, Howard RL, Smiddy NM, *et al.*: **Self-renewing monolayer of primary colonic or rectal epithelial cells**. *Cell Mol Gastroenterol Hepatol* 2017, **4**:165–182 e167.

70. Gunasekara DB, Speer J, Wang Y, Nguyen DL, Reed MI, Smiddy NM, Parker JS, Fallon JK, Smith PC, Sims CE, *et al.*: **A monolayer of primary colonic epithelium generated on a scaffold with a gradient of stiffness for drug transport studies**. *Anal Chem* 2018, **90**:13331–13340.

71. Nikolaev M, Mitrofanova O, Broguiere N, Geraldo S, Dutta D, Tabata Y, Elci B, Brandenberg N, Kolotuev I, Gjorevski N, *et al.*: **Homeostatic mini-intestines through scaffold-guided organoid morphogenesis**. *Nature* 2020, **585**:574–578.

72. * Ramalho AS, Furstova E, Vonk AM, Ferrante M, Verfaillie C, Dupont L, Boon M, Proesmans M, Beekman JM, Sarouk I, *et al.*: **Correction of CFTR function in intestinal organoids to guide treatment of cystic fibrosis**. *Eur Respir J* 2021, **57**:1902426.

This study examined modulator rescue of mutant CFTR in organoids generated from rectal biopsies of 97 pwCF. The results showed a correlation between in vitro CFTR function and clinical improvement in pwCF as measured by sweat chloride and lung function, demonstrating the relevance of testing CFTR modulators to rescue rare CFTR mutations in patient-derived rectal biopsies.

73. * Berkers G, van Mourik P, Vonk AM, Kruisselbrink E, Dekkers JF, de Winter-de Groot KM, Arets HGM, Marck-van der Wilt REP, Dijkema JS, Vanderschuren MM, *et al.*: **Rectal organoids enable personalized treatment of cystic fibrosis**. *Cell Rep* 2019, **26**: 1701–1708 e1703.

This study uses rectal organoids to demonstrate a correlation between in vitro readout of CFTR drug rescue and clinical improvement of pwCF. In addition, patient-derived stem cell cultures can be used as a model

to identify treatments for pwCF and can be biobanked as a resource for identifying personalized drug treatments.

74. Crawford DK, Mullenders J, Pott J, Boj SF, Landskroner-Eiger S, Goddeeris MM: **Targeting G542X CFTR nonsense alleles with ELX-02 restores CFTR function in human-derived intestinal organoids**. *J Cyst Fibros* 2021, **20**:436–442.

75. Geurts MH, de Poel E, Amatngalim GD, Oka R, Meijers FM, Kruisselbrink E, van Mourik P, Berkers G, de Winter-de Groot KM, Michel S, *et al.*: **CRISPR-based adenine editors correct nonsense mutations in a cystic fibrosis organoid biobank**. *Cell Stem Cell* 2020, **26**:503–510 e507.

76. Zomer-van Ommen DD, de Poel E, Kruisselbrink E, Oppelaar H, Vonk AM, Janssens HM, van der Ent CK, Hagemeijer MC, Beekman JM: **Comparison of ex vivo and in vitro intestinal cystic fibrosis models to measure CFTR-dependent ion channel activity**. *J Cyst Fibros* 2018, **17**:316–324.

77. Arora K, Yang F, Brewington J, McPhail G, Cortez AR, Sundaram N, Ramananda Y, Ogden H, Helmrath M, Clancy JP, *et al.*: **Patient personalized translational tools in cystic fibrosis to transform data from bench to bed-side and back**. *Am J Physiol Gastrointest Liver Physiol* 2021, **320**: G1123–G1130.

78. ** Muilwijk D, de Poel E, van Mourik P, Suen SWF, Vonk AM, Brunsveld JE, Kruisselbrink E, Oppelaar H, Hagemeijer MC, Berkers G, *et al.*: **Forskolin-induced organoid swelling is associated with long-term CF disease progression**. *Eur Respir J* 2022.

This publication compared intestinal organoid swelling assay data with long-term cystic fibrosis disease progression, as measured by patients' clinical data such as lung function, pancreatic insufficiency, liver disease, diabetes, and sweat chloride concentration.

79. * Shik Mun K, Arora K, Huang Y, Yang F, Yarlagadda S, Ramananda Y, Abu-El-Haija M, Palermo JJ, Appakalai BN, Nathan JD, *et al.*: **Patient-derived pancreas-on-a-chip to model cystic fibrosis-related disorders**. *Nat Commun* 2019, **10**:3124.

A pancreas-on-a-chip model was developed using 2 pancreatic cell types: ductal epithelial cells and islets. This model allows testing of CFTR modulators for their ability to improve glucose imbalances in pwCF that have CF-related diabetes.

80. Bijvelds MJC, Roos FJM, Meijsen KF, Roest HP, Verstegen MMA, Janssens HM, van der Laan LJW, de Jonge HR: **Rescue of chloride and bicarbonate transport by elexacaftor-ivacaftor-tezacaftor in organoid-derived CF intestinal and cholangiocyte monolayers**. *J Cyst Fibros* 2021.

81. Verstegen MMA, Roos FJM, Burka K, Gehart H, Jager M, de Wolf M, Bijvelds MJC, de Jonge HR, Ardisasmita AI, van Huizen NA, *et al.*: **Human extrahepatic and intrahepatic cholangiocyte organoids show region-specific differentiation potential and model cystic fibrosis-related bile duct disease**. *Sci Rep* 2020, **10**:21900.

82. Brennan LC, O'Sullivan A, MacLoughlin R: **Cellular therapy for the treatment of paediatric respiratory disease**. *Int J Mol Sci* 2021, **22**:8906.

83. Firth AL, Menon T, Parker GS, Qualls SJ, Lewis BM, Ke E, Dargitz CT, Wright R, Khanna A, Gage FH, *et al.*: **Functional gene correction for cystic fibrosis in lung epithelial cells generated from patient iPSCs**. *Cell Rep* 2015, **12**:1385–1390.

84. Chen YW, Huang SX, de Carvalho A, Ho SH, Islam MN, Volpi S, Notarangelo LD, Ciancanelli M, Casanova JL, Bhattacharya J, *et al.*: **A three-dimensional model of human lung development and disease from pluripotent stem cells**. *Nat Cell Biol* 2017, **19**: 542–549.

85. Hawkins F, Kramer P, Jacob A, Driver I, Thomas DC, McCauley KB, Skvir N, Crane AM, Kurmann AA, Hollenberg AN, *et al.*: **Prospective isolation of NKX2-1-expressing human lung progenitors derived from pluripotent stem cells**. *J Clin Invest* 2017, **127**:2277–2294.

86. Miller AJ, Hill DR, Nagy MS, Aoki Y, Dye BR, Chin AM, Huang S, Zhu F, White ES, Lama V, *et al.*: **In vitro induction and in vivo engraftment of lung bud tip progenitor cells derived from human pluripotent stem cells**. *Stem Cell Reports* 2018, **10**: 101–119.

87. ** Sone N, Konishi S, Igura K, Tamai K, Ikeo S, Korogi Y, Kanagaki S, Namba T, Yamamoto Y, Xu Y, *et al.*: **Multicellular modeling of ciliopathy by combining iPS cells and microfluidic airway-on-a-chip technology**. *Sci Transl Med* 2021, **13**: eabb1298.

This study is particularly important for modeling the cystic fibrosis airways as ciliopathy is a critical issue in diseased CF lungs.

88. ** Jiang JX, Wellhauser L, Laselva O, Utkina I, Bozoky Z, Gunawardena T, Ngan Z, Xia S, Di Paola M, Eckford PDW, *et al.*: **A new platform for high-throughput therapy testing on iPSC-derived lung progenitor cells from cystic fibrosis patients**. *Stem Cell Reports* 2021, **16**:2825–2837.

This study used lung progenitor cells from iPSCs to test drug rescue of mutant CFTR function in a high-throughput platform. This method may be particularly beneficial for pwCF with rare CFTR mutations that do not yet have approved CFTR-targeting therapies.

89. Wiedenmann S, Breunig M, Merkle J, von Toerne C, Georgiev T, Moussus M, Schulte L, Seufferlein T, Sterr M, Lickert H, *et al.*: **Single-cell-resolved differentiation of human induced pluripotent stem cells into pancreatic duct-like organoids on a microwell chip**. *Nat Biomed Eng* 2021, **5**:897–913.

90. Klaka P, Grudl S, Banowski B, Giesen M, Sattler A, Proksch P, Welss T, Forster T: **A novel organotypic 3D sweat gland model with physiological functionality**. *PLoS One* 2017, **12**, e0182752.

91. McMahon DB, Carey RM, Kohanski MA, Tong CCL, Papagiannopoulos P, Adappa ND, Palmer JN, Lee RJ: **Neuropeptide regulation of secretion and inflammation in human airway gland serous cells**. *Eur Respir J* 2020, **55**:1901386.

92. Leite SB, Roosens T, El Taghdouini A, Mannaerts I, Smout AJ, Najimi M, Sokal E, Noor F, Chesne C, van Grunsven LA: **Novel human hepatic organoid model enables testing of drug-induced liver fibrosis in vitro**. *Biomaterials* 2016, **78**:1–10.

93. * Lee RE, Miller SM, Mascenik TM, Lewis CA, Dang H, Boggs ZH, Tarran R, Randell SH: **Assessing human airway epithelial progenitor cells for cystic fibrosis cell therapy**. *Am J Respir Cell Mol Biol* 2020, **63**:374–385.

Cell therapy is being explored as an approach to treat CF that is independent of the CFTR mutation. In this study, HBE cells were tested for their suitability for CF cell therapy utilizing a repopulation assay. It was found that HBE expanded by the conditionally reprogrammed cell method most effectively displayed CFTR activity, and therefore may be useful for cell therapy for pwCF.

94. * O'Toole GA, Crabbe A, Kummerli R, LiPuma JJ, Bomberger JM, Davies JC, Limoli D, Phelan VV, Bliska JB, DePas WH, *et al.*: **Model systems to study the chronic, polymicrobial infections in cystic fibrosis: current approaches and exploring future directions**. *mBio* 2021, **12**, e0176321.

This publication is a comprehensive study on the effects of microbes in the CF lung, which is important for developing new model systems for discovering improved therapeutics.

95. ** Plebani R, Potla R, Soong M, Bai H, Izadifar Z, Jiang A, Travis RN, Belgur C, Dinis A, Cartwright MJ, *et al.*: **Modeling pulmonary cystic fibrosis in a human lung airway-on-a-chip: cystic fibrosis airway chip**. *J Cyst Fibros* 2021.

As a novel model of the CF lung, a CF Airway Chip was created that uses CF HBE grown at air-liquid interface and includes lung endothelium and dynamic fluid flow to deliver immune cells. These CF Airway Chips accurately represent the human airway in vivo, allowing direct testing of various drugs to rescue rare CFTR mutations.

96. Ogden HL, Kim H, Wikenheiser-Brokamp KA, Naren AP, Mun KS: **Cystic fibrosis human organs-on-a-chip**. *Micromachines (Basel)* 2021, **12**:747.

97. Rajan SAP, Aleman J, Wan M, Pourhabibi Zarandi N, Nzou G, Murphy S, Bishop CE, Sadri-Ardekani H, Shupe T, Atala A, *et al.*: **Probing prodrug metabolism and reciprocal toxicity with an integrated and humanized multi-tissue organ-on-a-chip platform**. *Acta Biomater* 2020, **106**:124–135.