



Review

Cystic fibrosis: Toward personalized therapies[☆]



Pauline T. Ikpa, Marcel J.C. Bijvelds, Hugo R. de Jonge^{*}

Erasmus MC-University Medical Center Rotterdam, Department of Gastroenterology & Hepatology, Rotterdam, The Netherlands

ARTICLE INFO

Article history:

Received 27 December 2013
Received in revised form 10 February 2014
Accepted 12 February 2014
Available online 20 February 2014

Keywords:

Cystic fibrosis
Personalized medicine
Organoids
CFTR correctors
CFTR potentiators

ABSTRACT

Cystic fibrosis (CF), the most common, life-threatening monogenetic disease in Caucasians, is caused by mutations in the CFTR gene, encoding a cAMP- and cGMP-regulated epithelial chloride channel. Symptomatic therapies treating end-organ manifestations have increased the life expectancy of CF patients toward a mean of 40 years. The recent development of CFTR-targeted drugs that emerged from high-throughput screening and are capable of correcting the basic defect promises to transform the therapeutic landscape from a trial-and-error prescription to personalized medicine. This stratified approach is tailored to a specific functional class of mutations in CFTR, but can be refined further to an individual level by exploiting recent advances in *ex vivo* drug testing methods. These tests range from CFTR functional measurements in rectal biopsies donated by a CF patient to the use of patient-derived intestinal or pulmonary organoids. Such organoids may serve as an inexhaustible source of epithelial cells that can be stored in biobanks and allow medium- to high-throughput screening of CFTR activators, correctors and potentiators on the basis of a simple microscopic assay monitoring organoid swelling. Thus the recent breakthrough in stem cell biology allowing the culturing of mini-organs from individual patients is not only relevant for future stem cell therapy, but may also allow the preclinical testing of new drugs or combinations that are optimally suited for an individual patient.

This article is part of a Directed Issue entitled: Cystic Fibrosis: From o-mics to cell biology, physiology, and therapeutic advances.

© 2014 Elsevier Ltd. All rights reserved.

Contents

| | |
|---|-----|
| 1. Introduction | 193 |
| 2. Mutation-specific therapies | 193 |
| 2.1. DNA repair | 194 |
| 2.2. RNA repair | 194 |
| 2.3. CFTR protein therapy | 195 |
| 2.3.1. Repair of class I mutant CFTR | 195 |
| 2.3.2. Repair of conformational defects in the CFTR protein | 195 |
| 2.3.3. Structural and functional repair of F508del-CFTR and other class II mutant CFTRs | 195 |
| 2.3.4. Improvement of CFTR gating by potentiators | 196 |
| 3. Toward individualized therapies | 196 |
| 3.1.1. Testing CF therapeutics in rectal biopsies and rectal organoids | 196 |
| 3.1.2. Organoids from airway epithelium as tools for testing CF therapeutics | 198 |
| 3.1.3. Testing CF therapeutics in immune cells | 198 |
| 4. Summary and future prospects and challenges | 198 |
| References | 199 |

Abbreviations: CFTR, cystic fibrosis transmembrane conductance regulator; rAAV, recombinant adeno-associated virus; NBD, nucleotide binding domain; MSD, membrane spanning domain; iPSC, induced pluripotent stem cells; CRC, conditionally reprogrammed cells; ROCK, Rho-kinase; ICM, intestinal current measurements; FIS, forskolin-induced swelling; PDE, phosphodiesterase; CAL, Golgi localized CFTR associated ligand; NHERF, Na⁺/H⁺ Exchanger Regulatory Factor; Fsk, forskolin; NMD, nonsense-mediated mRNA decay; PTCs, premature termination codons; PRs, proteostasis regulators; PCs, pharmacological chaperones; TMA, trimethylangelicin; HTS, high-throughput screening; ABPs, ATP binding pockets; WT, wild-type; FIS, forskolin-induced swelling; ALI, air-liquid interface; EMA, European Medicines Agency; FDA, Food & Drug Administration.

[☆] This article is part of a Directed Issue entitled: Cystic Fibrosis: From o-mics to cell biology, physiology, and therapeutic advances.

^{*} Corresponding author at: Department of Gastroenterology and Hepatology Erasmus MC, s'Gravendijkwal 230, NL-3015 CE Rotterdam, The Netherlands.

Tel.: +31 107031492; fax: +31 107032793.

E-mail address: h.dejonge@erasmusmc.nl (H.R. de Jonge).

1. Introduction

Cystic fibrosis (CF) is a life-threatening autosomal recessive monogenetic disease caused by mutations in the CFTR gene, encoding a cyclic AMP- and cyclic GMP-regulated and ATP-gated chloride channel (Riordan, 2008). The impact of a defect in CFTR function differs among tissues and cell types (Antunovic et al., 2013). In sweat glands, the decreased reabsorption of sodium chloride by the water-impermeable ductal epithelium results in elevated sweat chloride, a hallmark of CF. In other epithelia, in particular the respiratory and intestinal epithelia as well as the biliary and pancreatic ducts, CFTR dysfunction causes a loss of chloride and bicarbonate secretion, resulting in cellular alkalinity and luminal acidification, impaired decondensation of discharged mucin granules by goblet cells, and defective mucus expansion (De Lisle and Borowitz, 2013; Greggio et al., 2013). The ensuing acidification and dehydration of the mucus layer leads to impaired mucociliary clearance and bacterial killing by epithelial defensins (Pezzulo et al., 2012); this predisposes to recurrent infection, inflammation, mucus plugging and luminal obstruction. Loss of CFTR function in monocytes and macrophages, by impairing phagocytosis and intracellular killing of *Pseudomonas aeruginosa*, contributes to the enhanced susceptibility to infection in patients with CF (Bonfield et al., 2012; Sorio et al., 2011; Van de Weert-van Leeuwen et al., 2013).

When CF was first described in 1938, the predicted survival age of a CF patient was only 6 months. For patients born in the 1990's median survival is now predicted to exceed 40 years (Wilschanski, 2013). This impressive gain in life expectancy has resulted largely from advances in early diagnosis and symptomatic treatment of end-organ pathologies based on vast improvements in nutrition, control of airway infections, and physiotherapy (see Fig. 1 for an overview of CF symptomatic treatment). Understandably the discovery of the disease-causing CFTR gene in 1989 created new hope for a curative treatment targeting the basic defect rather

than treating CF disease manifestations (Clancy and Jain, 2012). The most obvious approach, viral or non-viral gene therapy, would potentially be of benefit to all patients with CF, independent of their genotype. So far however CFTR gene addition or gene replacement, despite promising advances, has not translated into clinical benefits despite more than 20 clinical trials, mainly due to a low expression of the CFTR transgene, inflammatory responses to viral proteins, the development of a humoral immune response preventing successful readministration, and the risk of insertional mutagenesis in case of integrating viral vectors. In contrast, considerable progress has been made in the development of tailored CFTR pharmacotherapy for specific CFTR mutations, and the design of better *in vitro* preclinical assays that allow the selection of the most effective therapeutic approaches on an individual basis, i.e. personalized medicine.

2. Mutation-specific therapies

Nearly 2000 mutations in the CFTR gene have been identified that can be subdivided in 6 different classes based on their phenotypic consequences (Fig. 2) (Derichs, 2013; Rogan et al., 2011). Class I mutations cause defects in full-length protein synthesis due to premature truncations or nonsense alleles, or to severe splicing defects; class II mutations cause folding defects and premature proteasomal degradation, class III shows normal trafficking to the plasma membrane but defective channel gating, class IV results in an impaired channel conductance, class V causes a reduced number of CFTR transcripts, and class VI is characterized by a reduced protein stability and increased turnover of CFTR at the cell surface. The first three mutation classes are associated with a nearly complete loss of CFTR channel function and are considered severe mutations, whereas mutations in class IV–VI may allow residual CFTR function and are associated with a milder phenotype. F508del, the most common mutation with an allelic frequency of around 90% worldwide, has mixed properties of class II, III and VI and is therefore most

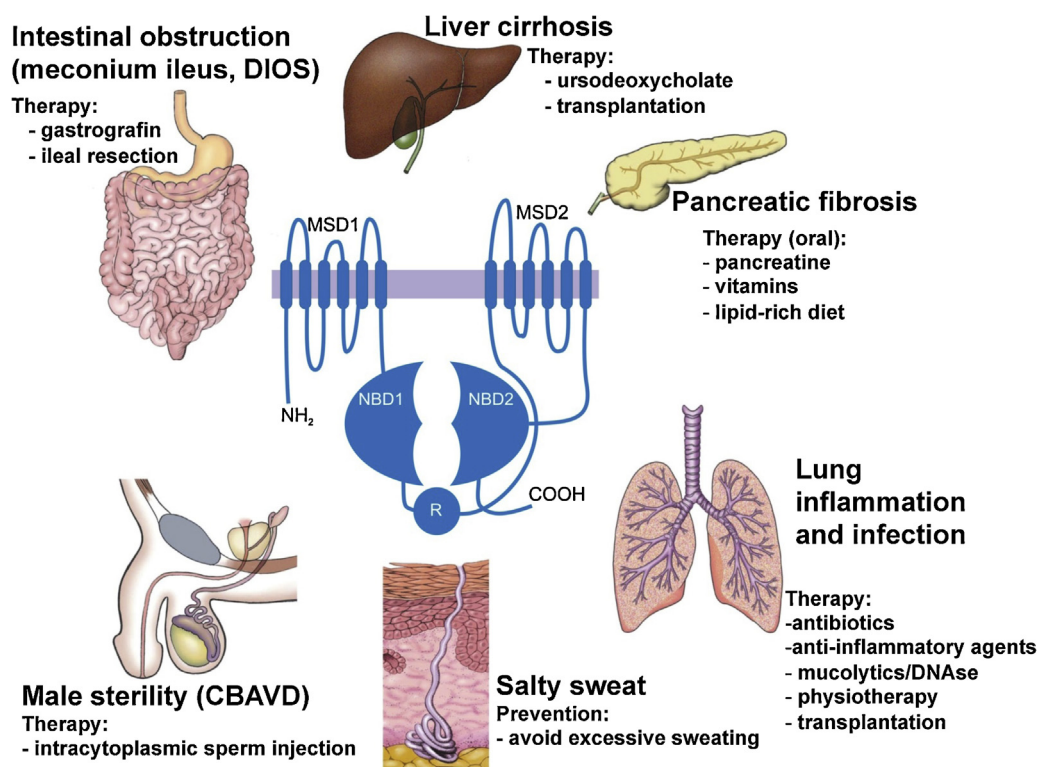


Fig. 1. Overview of current symptomatic treatments of CF. Inset: CFTR domain structure. MSD, membrane spanning domain; NBD, nucleotide binding domain; R, regulatory domain.

CFTR: >1900 MUTATIONS, 6 MUTATION CLASSES (I–VI)

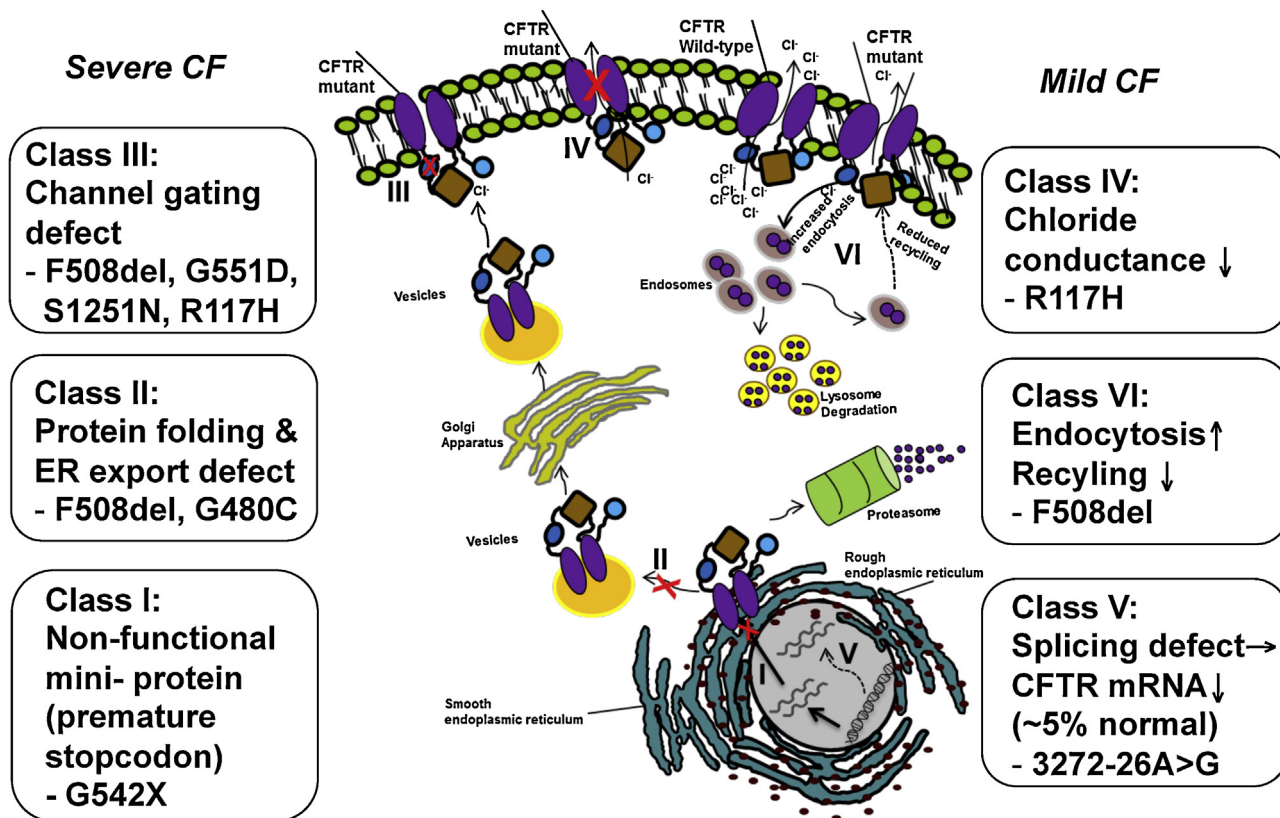


Fig. 2. Overview of CFTR mutation classes. The functional consequences of the mutations are indicated in the flanking boxes.

difficult to repair at the protein level. Just four other mutations, notably G551D (class III), W1282X, G542X (class I), and N1303K (class II) have a worldwide prevalence of 1–3% each, whereas only 20 mutations have a frequency above 0.1%.

Before the introduction of high-throughput screening (HTS) as a new technique in CFTR drug discovery, proof of principle showing that mutation-specific repair of CFTR is feasible was reached at the DNA, RNA and protein level. A major advantage of this approach as compared to gene addition by cDNA vectors is that only those cells expressing CFTR endogenously are corrected. Potential deleterious consequences of ectopic expression of the CFTR transgene, such as supraphysiological expression of CFTR in the cells not normally expressing CFTR are avoided, and the need for CFTR promoter constructs or CFTR minigenes that are difficult to transfect and to integrate into viral vectors, is abolished.

2.1. DNA repair

One promising approach to reach mutation-specific repair of CFTR at the DNA level is the application of recently developed genomic editing techniques, such as the Zinc-finger nuclease (ZFN), TAL effector nucleases (TALENs), or CRISPR/Cas9 system for genome engineering (Joung and Sander, 2013; Lee et al., 2012). Proof of concept for correction of the F508del mutation in exon 11 by homologous recombination with a donor plasmid encoding wild-type CFTR sequences using CRISPR/Cas9 has been reached recently in human intestinal stem cells (Schwank et al., 2013). In principle this approach is capable of correcting most if not all other “severe” class I–III mutations, by proper engineering of the 20 nucleotide guide sequence in the single guide RNA that targets the Cas9

nuclease to appropriate cleavage sites. However its direct therapeutic application in CF patients *in vivo* is hampered by the low efficacy of the repair and the mutagenic risk, requiring a time-consuming selection step of the repaired cells that is not feasible *in vivo*.

2.2. RNA repair

Partial transcriptional repair of F508del-CFTR and functional restoration of chloride transport in human CF airway cells has been reached by spliceosome-mediated RNA *trans*-splicing technology (SMArt) using recombinant adenovirus or adeno-associated virus (rAAV) as the delivery vector (Liu et al., 2005; Mansfield et al., 2003). Alternatively, insertion of the missing bases (TTT) by hybridization to a 2'-O-methyl RNA-unmodified RNA oligonucleotide duplex has been achieved in a F508del-CFTR expressing cell line (Zamecnik et al., 2004). Although RNA repair is transient and only modestly effective, studies of CFTR splicing polymorphisms suggest that 8% of normal CFTR message is sufficient for normal lung function, whereas 5% is associated with relatively mild CF lung disease (Chu et al., 1992; Ramalho et al., 2002). Genetic reprogramming of CFTR transcripts by SMArt or oligonucleotides may in principle be applicable also to other severe CFTR mutations. In addition, the disease-causing effect of CFTR mutations affecting the pre-mRNA splicing of the CFTR gene by disrupting or generating intronic splicing motifs (frequency ~13%), may be reduced in a mutation-specific fashion by bifunctional antisense oligonucleotides providing a *trans*-acting splicing enhancer, or by synthetic exon-specific activators or modified U1 small nuclear RNAs capable of correcting exon skipping (Fernandez Alanis et al., 2012; Nissim-Rafinia et al., 2004). However the clinical utility of these techniques

for ameliorating CF lung disease is not yet proven and awaits the development of suitable and safe *in vivo* delivery vectors, for example rAAV engineered to escape neutralizing antibodies, or methods to penetrate the mucus and periciliary layer in the CF lung more effectively (Hida et al., 2011). Because oligonucleotide and viral repair techniques are less suitable for oral or systemic delivery, these therapies are likely to remain restricted to aerosolic treatment which targets only the lung compartment, in contrast to the orally applied CF therapeutics discussed hereafter.

2.3. CFTR protein therapy

2.3.1. Repair of class I mutant CFTR

Of all CFTR mutations identified, ~10% are nonsense mutations (class I) that result in the creation of premature termination codons (PTCs) and rapid degradation of CFTR transcripts by a quality control mechanism known as nonsense-mediated mRNA decay (NMD) to prevent the synthesis of truncated proteins with potential dominant negative effects (Clancy and Jain, 2012; Derichs, 2013; Wilschanski, 2013). Aminoglycoside antibiotics, including tobramycin, gentamycin and amikacin, do not repair the RNA directly but bind to the ribosomes and cause the insertion of a near cognate amino-acyl tRNA into the ribosomal A site. This process suppresses translational fidelity and allows the ribosome to read through the PTC and to produce full-length protein.

Recently the non-aminoglycoside PTC suppressor ataluren (PTC 124; identified by HTS) which is orally bioavailable and non-toxic, has been assessed in a large phase III clinical trial. Despite some success in Phase II testing (Sermet-Gaudelus et al., 2010; Wilschanski et al., 2011), ataluren did not significantly improve the primary endpoint in Phase III (Xue et al., 2013). Several recent *in vitro* reporter assay studies likewise failed to confirm the read-through efficacy of PTC124, and the reliability of the original HTS has been questioned (McElroy et al., 2013). However encouraging results were obtained recently in preclinical studies using a second generation of synthetic aminoglycosides designed to provide higher readthrough activity with reduced mitochondrial toxicity (Xue et al., 2013).

2.3.2. Repair of conformational defects in the CFTR protein

Defects in protein conformation, as occur in mutant CFTR, can be rescued by two different approaches: (1) through stabilizing the protein native state with pharmacological chaperones (PCs) that bind directly to the channel protein; and (2) through altering the activity of the transcriptional, folding, or membrane trafficking machinery or blocking the degradation of partially folded, but functional CFTR at the endoplasmic reticulum (ER) or the plasma membrane (PM) by so-called proteostasis regulators (PRs) (Lukacs and Verkman, 2012; Okuyoneda and Lukacs, 2012). Most CFTR trafficking mutants, including F508del, with a few exceptions (e.g. G480C; N287Y), show additional defects in channel gating function, and most PCs and PRs identified thus far only partially normalize the channel conformational defect. Effective protein repair may therefore need the combined use of either PCs or PRs (collectively called ‘correctors’) which promote CFTR exit from the ER and accumulation in the PM, and ‘potentiators’ which improve channel gating (Rowe and Verkman, 2013).

2.3.3. Structural and functional repair of F508del-CFTR and other class II mutant CFTRs

It has recently been shown that F508del-CFTR actually contains two distinct folding defects. Studies on second site suppressor mutations and evolved sequences coupled to the F508del residue showed not only misfolding of NBD1 (containing residue 508), but also instability of the NBD1-MSD2 interface (Mendoza et al., 2012; Rabeh et al., 2012). This may explain the rather modest rescue effect

of most CFTR correctors available to date, which target only a single defect. Thus multidrug therapy with a combination of a NBD1 domain stabilizer (now mechanistically classified as a “class I corrector”) and a NBD1-MSD2 interface stabilizer (“class II corrector”) may be required to overcome the previously observed “ceiling” in CFTR protein repair (Okuyoneda et al., 2013). Conceivably, the parallel targeting of multiple conformational defects by separate small molecule correctors will allow wild-type folding of the mutant protein and obviate the need for a potentiator.

The current collection of preclinically active PCs and PRs is broad and rapidly expanding. It includes not only small molecules but also transcomplementing CFTR fragments (Cormet-Boyaka et al., 2004) and RXR peptides (Kim Chiaw et al., 2009). The most promising PC developed so far, VX-809 (Lumacaftor; Vertex Pharmaceuticals) has emerged from HTS in a recombinant cell-based assay and is capable of restoring ~15% CFTR channel activity in primary respiratory epithelia expressing F508del-CFTR (Van Goor et al., 2011). VX-809 is more selective for CFTR than most other folding correctors (for example VRT-325 and corr-4a) and acts early in CFTR biogenesis to interact directly with MSD1 and modulate its conformation (Loo et al., 2013; Ren et al., 2013). In this way it not only corrects disease-causing mutations in CFTR MSD1 with a high efficacy, but also allosterically suppresses the assembly defects in CFTR caused by F508del and by several other remote folding mutations in the fourth intracellular loop (ICL4). Therefore VX-809 mono- or combination therapy, by promoting interdomain communication, may restore function to a large number of rare CFTR mutations, aside its main action as a class II F508del-CFTR corrector.

Results of a phase IIa clinical study of VX-809 showed a small but significant reduction of sweat chloride values, but no significant improvement in nasal potential difference (NPD), lung function or maturation of F508del-CFTR in rectal biopsies (Clancy et al., 2012). VX-809 is reported to normalize the gating of corrected F508del-CFTR but has no direct potentiating action, explaining why acute addition of a potentiator further increased the activity of VX-809-corrected F508del-CFTR (as well as wild-type CFTR) by two-fold (Van Goor et al., 2011). The therapeutic relevance of this gain in activity is presently explored in a phase III clinical study of homozygous F508del CF patients, encouraged by data from a phase II study showing improved lung function in approximately 50% of the participants undergoing VX-809/VX-770 combi-therapy (see <http://www.cff.org>).

Aside VX-809, several other small molecule F508del-CFTR correctors may qualify as candidate CF therapeutics, some of which are already in clinical use against other diseases. These include the psoralen-related compound and psoriasis drug trimethylangelicin (TMA), granted orphan designation by the EMA, and acting as a “dual” F508del-CFTR corrector and potentiator, and as a suppressor of IL-8 transcription (Tamanini et al., 2011); the cyanoquinoline CoPo-22 emerging from HTS, with dual corrector and potentiator activities albeit in the micromolar range (Phuan et al., 2011); *in silico*-selected disruptors of keratin8-F508del NBD1 interaction, functioning as potent correctors of CFTR maturation and trafficking (Odolczyk et al., 2013); inhibitors of F508del CFTR-CAL interaction, promoting the formation of a stable F508del CFTR-NHERF1 complex in the plasma membrane (Wolde et al., 2007); inhibitors of the Hsp90 co-chaperone Aha1 (Koulov et al., 2010); and sildenafil, a phosphodiesterase type V (PDE5) inhibitor used clinically against erectile dysfunction (Dhooghe et al., 2013; Leier et al., 2012; Lubamba et al., 2008; Noel et al., 2012). Sildenafil may increase CFTR activity through cGMP-dependent phosphorylation or through direct cGMP-CFTR interaction, but is also functioning as a relatively weak F508del-CFTR corrector, possibly due to its structural resemblance with the corrector VRT-325 (Kalid et al., 2010). Although the rapid expansion of a broad collection of CFTR correctors is encouraging, in most cases additional mechanistic and/or

toxicity studies remain to be done before clinical testing can be considered.

2.3.4. Improvement of CFTR gating by potentiators

Most CFTR class III gating mutations, including the most common “Keltic” mutation, G551D (frequency ~3%) are localized in the ABC signature sequence or in the Walker A or B motif of the two ATP binding pockets (ABP1 and ABP2) of the NBDs (cf. Fig. 1, center), and cause a defect in ATP-dependent channel gating without abnormalities in synthesis, trafficking or CFTR phosphorylation (Yu et al., 2011). Recent insights into the CFTR gating mechanism have revealed the existence of two distinct open states, O1 and O2, with different NBD configurations: a highly stable O1 state, in which with ATP occupies the ABP2 catalytic site, and the more transient O2 state, lacking ATP in ABP2 (Jih and Hwang, 2013). VX-770 (Ivacaftor), the first FDA- and EMA-approved CF medication forthcoming from HTS that directly targets CFTR, was found to stabilize the O2 state, thereby promoting ATP-independent gating. This non-conventional gating mechanism could be reproduced in purified G551D-CFTR inserted in planar lipid bilayers and proteoliposomes, confirming that VX-770 binds directly to the CFTR protein, most plausibly to the MSDs rather than the NBDs (Eckford et al., 2012). Stabilizing O2 may also promote reentry of the channel into the gating cycle and the transition to the O1 state, thereby increasing ATP-dependent gating of WT-CFTR. This new gating model may explain why VX-770 increases the open probability of both WT CFTR and all 10 class III gating mutants tested (Yu et al., 2012), as well as numerous other CFTR forms with missense mutations or deletions, including F508del and R117H (Rowe and Verkman, 2013; Van Goor et al., 2014).

In several phase III trials oral VX-770 treatment of G551D patients led to rapid, dramatic and sustained improvements in FEV1 and body weight, and a strong reduction of sweat chloride and pulmonary exacerbations (reviewed in Rowe and Verkman, 2013). However a 4-month placebo-controlled trial of Ivacaftor in F508del-CFTR homozygous patients only reduced sweat chloride by a small amount (Flume et al., 2012). The relative failure of VX-770 monotherapy in F508del patients has been commonly attributed to the very low levels of F508del-CFTR at the cell surface. However recent studies on human bronchial cells suggest an alternative explanation: in these cells, chronic exposure to VX-770 resulted in a dramatic increase in endocytosis and lysosomal degradation of WT- and F508del-CFTR, but not of G551D channels (Cholon DM, 2013). Whether this phenomenon is limited to VX-770 or is a property of other potentiators too, is not known and warrants further investigation. If occurring also *in vivo*, it may impact the outcome of the ongoing phase III combi-trial of VX-809 and VX-770.

The isoflavone genistein, a major component of soya-rich food, potentiates G551D- and F508del-CFTR channels by binding to the NBDs and promoting ATP-dependent gating (French et al., 1997; Sohma et al., 2013), but also enhances CFTR phosphorylation at basal cAMP levels (Pyle et al., 2011) and inhibits CFTR endocytosis through an ill-defined mechanism (Lim et al., 2007). In contrast, curcumin, another food component and CFTR potentiator, mimics the effect of VX-770 by binding to the MSDs and promoting ATP-independent channel gating (Wang et al., 2007). As predicted from their distinct mechanism of action, the combined application of genistein and curcumin in a low concentration range (5–10 μ M) synergistically restored the gating defect of G551D of up to ~50% of the WT level (Sohma et al., 2013). Further clinical studies using combinations of both food components (or combinations of genistein and VX-770, likewise predicted to act synergistically) are needed to determine the therapeutic potential of the combination therapy relative to monotherapy.

3. Toward individualized therapies

The success of VX-770 monotherapy in G551D patients and improved insights into the mechanism of action of CFTR potentiators and correctors have greatly boosted attempts to develop more potent and specific CFTR modulators targeting a specific class of mutant CFTR. To define the optimal treatment for an individual patient, genotyping of CFTR is clearly the first step. Subsequently, in case of rare mutations with an unknown phenotype, one approach is to express the protein from a mutated cDNA in a model cell line, for example Fischer rat thyroid cells (Van Goor et al., 2013), followed by CFTR localization and functional assays in the absence and presence of CFTR modulators. This method however is unable to report possible differences in corrector and potentiator response among individuals belonging to the same CFTR mutation class, such as caused by polymorphisms in the CFTR gene and CF modifier genes, or by differences in the CFTR interactome. Moreover this technique may be prone to artifacts inherent to CFTR overexpression or to the heterologous cellular background, and is unsuitable for studying splice mutations or complex genotypes, e.g. CF compound heterozygotes. Such confounding factors may explain why some correctors potentially act in transfected cell lines but fail in primary epithelial cell cultures (Pedemonte et al., 2010).

Considering the limitations of heterologous expression systems, the use of native epithelium or primary cell cultures, obtained from CF patients, for CFTR corrector testing seems indicated. Evidently, access to such patient material is limited, and this has hampered its application for this purpose. However recent breakthroughs in the field of stem cell biology now allow large scale cell expansion of non-transformed cells, derived from single biopsies (skin, intestine, respiratory tissue) or blood samples, opening a new chapter for translational CF research.

3.1.1. Testing CF therapeutics in rectal biopsies and rectal organoids

As illustrated in Fig. 3, one convenient and minimally invasive technique that is even applicable to CF neonates starts with the harvest of one or more rectal biopsy specimens by forceps or suction, followed by measurements of CFTR-mediated transepithelial chloride and bicarbonate currents (“ICM”) in Ussing chambers (Clancy et al., 2013; De Boeck et al., 2011; De Jonge et al., 2004). Both acute and chronic effects of CFTR modulators can be monitored using incubation conditions that maintain tissue viability for at least 20 h. Uniquely this technique allows testing of CFTR modulators *ex vivo* in native, non-cultured epithelium, in which, auspiciously, non-CFTR chloride channels minimally contribute to the ICM response. It can also be used to monitor other CF-relevant pathologies, such as intracellular alkalinization and defects in mucin degranulation. Studies on biopsies from class V splice mutation patients indicate that the level of CFTR expression in colonic epithelium is such that only circa 20% of mature, normal CFTR protein in this two-membrane assay (apical chloride exit; basolateral chloride import) suffices to normalize the ICM response (when CFTR is stimulated by saturating forskolin/cAMP levels). This implies that ICM can detect minute improvements in CFTR function. However, because ICM responses plateau at >20% of normal protein abundance, its dynamic range is rather narrow (De Boeck et al., 2011). To some extent, this range can be expanded by using suboptimal stimulation (low forskolin). Such a strategy may be particularly important to identify corrector effects in biopsies carrying very mild mutations (e.g. R117H) that are associated with <80% loss of CFTR function.

A clear limitation of the ICM test is its low capacity (only 4–8 biopsies/patient) and lack of expandability and suitability for large-scale corrector screening. However this problem can be

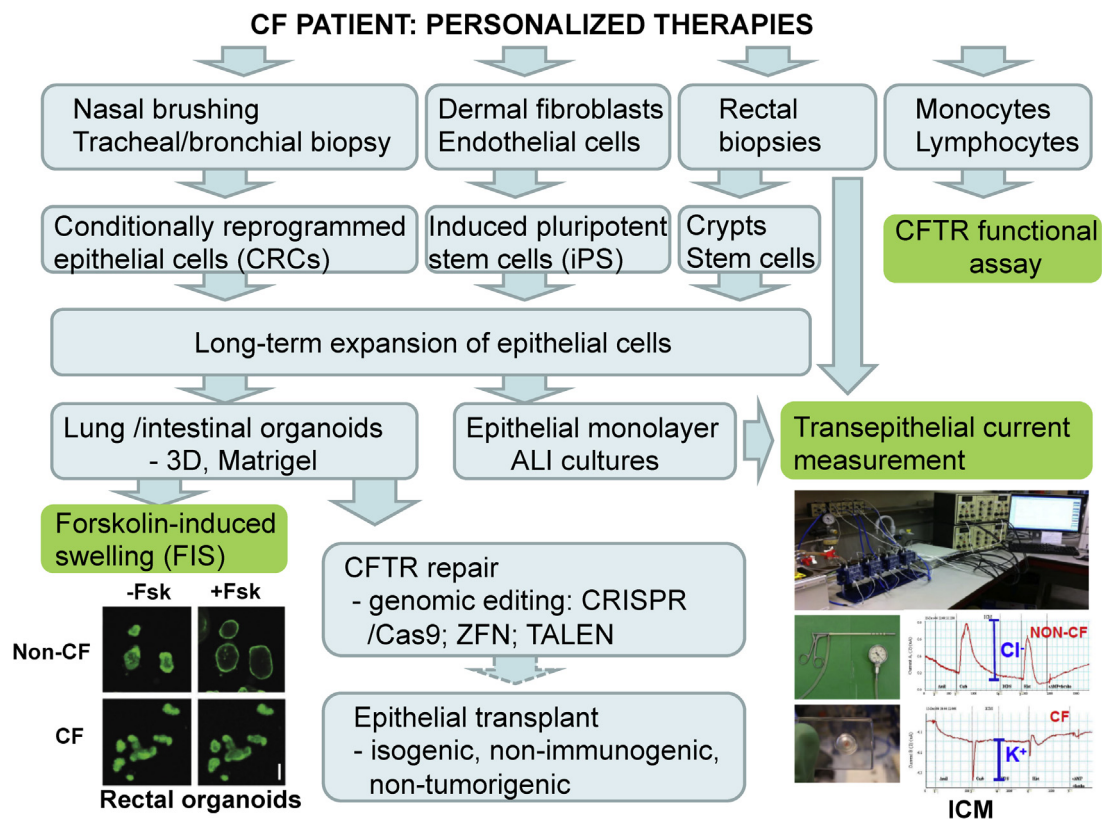


Fig. 3. Personalized medicine entails drug testing on cells or tissues obtained from individual CF patients to pinpoint the most effective treatment strategy.

Suitable cell types or tissues (i) express sufficient amounts of CFTR to allow large scale assessment of CFTR activity; (ii) are readily available from each patient (preferably also from infants) through a well-tolerated and safe isolation procedure; (iii) are expandable so to allow testing of multiple drug candidates within a wide concentration range and, preferably, at suboptimal and optimal cAMP levels (to mimic the variable hormonal/neuronal stimulation by cAMP agonists seen *in vivo*). The tissues/cell types selected for the flow diagram represent the major if not sole sources satisfying these criteria. The most effective (combination of) CFTR modulator(s) is selected on the basis of its performance in assays that (directly or indirectly) assess CFTR function (green boxes).

overcome by converting the rectal biopsies into epithelial organoids (also named enteroids) using a 3-D culturing technique developed recently by the Clevers group (Sato and Clevers, 2013; Sato et al., 2009, 2011). This primary culture method enables intestinal stem cells to expand into closed and self-renewing organoids containing crypt-like structures and an internal lumen lined by differentiated cells, recapitulating the *in vivo* tissue architecture. Activation of CFTR by the cAMP agonist forskolin drives the secretion of electrolytes and fluid into the lumen of the organoid, resulting in forskolin-induced swelling (FIS) (Dekkers et al., 2013). Luminal expansion is fully blocked by pharmacological CFTR inhibitors and completely absent from CFTR-null organoids, underscoring the validity of FIS as a CFTR-specific assay. Quantification of FIS in calcein green-labeled organoids grown in 96-well plates (up to 80 organoids/well) using live-cell confocal microscopy and imaging software appeared highly reproducible and allowed the testing of up to 32 conditions per experiment in triplicate. Normalized non-corrected FIS rates differed greatly between different CFTR genotypes (non-CF > class V (A455E) > class II (F508del) > class I), and, perhaps less evident, between individuals carrying the same alleles (*i.e.* F508del/F508del). Similar to the ICM, FIS is a two-membrane assay reaching a “ceiling” at about 20% of “normal” CFTR activity, beyond which apical CFTR activity no longer limits the rate of water secretion. As in case of ICM, the assay is highly sensitive which explains why organoids derived from homozygous F508del/F508del patients, without prior corrector treatment, show a definite residual, albeit variable FIS activity (~5% of non-CF, corresponding with ~1% CFTR activity), which can be enhanced by potentiators, such as VX-770 (Dekkers et al., 2013).

The FIS assay readily reveals that organoids isolated from different CF patients, even when these carry the same or similar (*i.e.* same class) CF alleles, may respond differently to (1) a single corrector or potentiator; (2) a combination of corrector and potentiator (*e.g.* VX-809 + VX-770 in F508del homozygotes), and (3) a combination of two different correctors (*e.g.* VRT-325 and Corr-4a) (Dekkers et al., 2013). Even larger individual differences are expected in case of a PR-type of CFTR corrector considering its stronger dependence on cellular background and CFTR interactome in comparison with a PC.

Obviously the FIS assay can only report *intrinsic* differences between CFTR modulator efficacies at the level of intestinal epithelial cells; it cannot account for individual differences in numerous other therapeutically relevant factors *in vivo*, such as pharmacokinetics, infection, inflammation, mesenchyme-epithelium and lymphocyte-epithelium communication, and the frequency and intensity of physiological stimuli, in particular β -adrenergic and VIPergic cAMP agonists. Therefore additional studies designed to correlate drug responses *in vivo* with drug testing by FIS in organoids from the same patient are needed to gain more insight into the predictive value of the FIS assay. Moreover, rectal biopsies are the most convenient source for generating intestinal organoids from individual CF patients, but the results of the FIS assay in rectal organoids may not always be representative for drug responses in other intestinal segments, in particular the small intestine.

Another favorable aspect of organoid cultures is their ability to maintain intact stem cell compartments and (epi)genetic stability even after >40 passages. Repetitive passaging will generate a large number of organoids from individual patients that can be

stored in biobanks and used later for testing newly developed CF therapeutics.

3.1.2. Organoids from airway epithelium as tools for testing CF therapeutics

Although the intestine and lungs both originate from endoderm during embryonic development, intestinal epithelium cannot be considered simply as a surrogate model for the airways. In contrast to the intestine, the cell turnover in the lungs is very low but accelerates upon tissue injury, causing cryptic stem cells (presumably basal cells) to self-renew and generate luminal cells, including differentiated ciliated cells (Rock et al., 2009). Similar to intestinal stem cells, these cells may form spheric structures *in vitro*. More recent studies have shown that this process is reversible, and that fully differentiated epithelial cells, as present in tracheal or bronchial biopsies, can be reprogrammed to reach an adult stem cell-like, indefinite proliferative state (Liu et al., 2012; Supryniewicz et al., 2012). Induction of these karyotype-stable and nontumorigenic “conditionally reprogrammed” cells (CRCs) is reached by isolating cells from small biopsies or cryopreserved tissue by protease dissociation and growing the cells on a layer of fibroblast feeder cells in the presence of a Rho kinase (ROCK) inhibitor. Importantly, these cells maintain their epigenetic memory and, following expansion, shift back to their original differentiated phenotype, including ciliated cells and goblet cells, upon removal of the feeders and the inhibitor. By placing them into an appropriate 3D culture system, they are capable of forming spheric lung organoids (grown in Matrigel) or monolayers of epithelial cells (grown as air-liquid interface cultures on filters). These can potentially be used to monitor CFTR activity in FIS assays or transepithelial current measurements, and to study the efficacy of CFTR modulators, in analogy to rectal biopsies and organoids (Fig. 3). Potential hurdles however are the relatively low expression levels of CFTR in human lung epithelial cells, possibly resulting in very low FIS rates and transepithelial currents, and the presence of compensatory non-CFTR channels, e.g. calcium-activated chloride channels, that are much less prominent in the intestine. Clearly further research is needed to evaluate the feasibility of CF drug testing in epithelial monolayers and organoids derived from nasal brushings or bronchial biopsies. If successful, this approach would offer the same advantages for individual drug testing as discussed for rectal organoids, but, considering the severe CF lung pathology, obtained in an even more CF-relevant cell type.

Another option to generate patient-specific airway epithelial cells for testing CF therapeutics starts with the creation of induced pluripotent stem cell (iPSC) lines from dermal fibroblasts donated by the CF patient, followed by an *in vitro* directed differentiation protocol using growth factors that mimic endoderm developmental pathways, and culturing the cells under Air-liquid-interface (ALI) conditions on permeable substrates (Wong et al., 2012). However, although a subpopulation of cells did express CFTR and showed CFTR activity in an iodide efflux assay, the cell culturing procedure was highly time-consuming, and the assay was not robust enough to demonstrate clear effects of a VX-809 like corrector.

3.1.3. Testing CF therapeutics in immune cells

The “personalized medicine” approaches discussed so far are restricted to epithelial cells and organoids; however, CFTR is also expressed at low but functionally important amounts in non-epithelial cells including myeloid cells (Bonfield et al., 2012), in particular monocytes (Sorio et al., 2011; Van de Weert-van Leeuwen et al., 2013), macrophages (Deriy et al., 2009) and lymphocytes (Krauss et al., 1992). CFTR defects in these cells may contribute to abnormalities in the innate immune defense of CF patients

(Tang et al., 2012), implying that restoration of CFTR function by orally available CFTR correctors and potentiators may be of prime importance in preventing inflammation and infection. Though our present knowledge of CFTR biosynthesis, processing and function in immune cells is fairly limited (Sorio et al., 2011), studying the effect of CFTR modulators in cells isolated from individual CF patients is clinically highly relevant. This can be done on a small scale using monocytes isolated freshly from blood samples of the patient, or following expansion of lymphocyte cell lines immortalized with Epstein–Barr virus, using fluorescent membrane potential sensing probes or other live assays to monitor CFTR activity (Fig. 3).

4. Summary and future prospects and challenges

The development of mutation-specific and personalized therapies for CF is progressing rapidly, spurred by major technical advances at various levels. Firstly, the introduction of reliable newborn screening methods allows early evaluation of the efficacy of CFTR-restoring drugs, and optimization of treatment at the personal level, before overt disease progression sets in. Secondly, the advent of next generation DNA sequencing techniques, which allow the rapid detection of mutations within the full length of the CFTR gene, including introns and the promoter region. Thirdly, the development by HTS and other approaches, of an ever-expanding collection of CFTR protein correctors and potentiators, with proof of clinical efficacy established for at least one compound, the CFTR potentiator VX-770. Due to the oral bioavailability of these new CF therapeutics, CFTR repair is no longer restricted to the lungs, as is the case for most DNA- and RNA-based mutation repair techniques, but is feasible in virtually all CF-relevant cell types, including bile ducts, intestine, sweat glands and immune cells.

Finally, the development of novel techniques to generate almost unlimited numbers of epithelial cells and organoids from stem cells in the intestine and from conditionally reprogrammed stem cells in the airways, and the introduction of simple, robust and high-throughput assays to measure CFTR activity in organoids by FIS, has made it possible to perform pre-clinical CFTR modulator screens in cells originating from individual CF patients. This approach is clearly superior to modulator assays in heterologous mutant CFTR expression systems because (1) it allows the detection of modulator responses for individual CF patients, irrespective of the mutation, and of possible differences in response to the same modulator between CF patients; (2) CFTR correction is assessed in a native cellular and genetic background, closely reflecting the *in vivo* condition; (3) artifacts of CFTR under- or overexpression are avoided. Moreover, the option to store cells or organoids from individual CF patients in biobanks implies that only a single donation of a small amount of tissue is needed to allow repetitive preclinical optimization of treatment with existing CF therapeutics or with new drugs emerging from drug development programs. It should be emphasized, however, that the value of the new preclinical drug screening tests for predicting the efficacy of a CF drug in the clinic still awaits thorough verification by comparisons of *in vitro* versus *in vivo* drug responses in a large number of CF patients belonging to different mutation classes.

Patient-specific epithelial organoids also hold promise for regenerative medicine approaches to treat CF disease in the lungs, intestine, and perhaps other organs including the exocrine and endocrine pancreas (Greggio et al., 2013; Huch et al., 2013b) and the liver (Huch et al., 2013a, 2013c) (Fig. 3). The feasibility of *in vivo* epithelial transplantation was demonstrated recently in a mouse model showing long-term (>6 months) repair of chemically-induced lesions in the colon of immunocompromised recipient mice by functional engraftment of colonic organoids expanded *in vitro* from a single adult colonic stem cell (Yui et al., 2012). By

combining this approach with mutation-specific repair of CFTR in the patient's own tracheal/bronchial organoids, as has been done successfully in human intestinal organoids using the CRISPR/Cas9 genome editing system, stepwise replacement of CF epithelium by isogenic, non-immunogenic and non-tumorigenic epithelial transplants may perhaps become feasible in the far future.

References

- Antunovic SS, Lukac M, Vujovic D. Longitudinal cystic fibrosis care. *Clin Pharmacol Ther* 2013;93:86–97.
- Bonfield TL, Hodges CA, Cotton CU, Drumm ML. Absence of the cystic fibrosis transmembrane regulator (Cftr) from myeloid-derived cells slows resolution of inflammation and infection. *J Leukoc Biol* 2012;92:1111–22.
- Cholon DM QN, Fulcher ML, Randell SH, Boucher RC, Gentzsch M. Potentiator ivacaftor impedes pharmacological correction of F508del CFTR. *Pediatr Pulmonol Suppl* 2013;36:225.
- Chu CS, Trapnell BC, Curristin SM, Cutting GR, Crystal RG. Extensive posttranscriptional deletion of the coding sequences for part of nucleotide-binding fold 1 in respiratory epithelial mRNA transcripts of the cystic fibrosis transmembrane conductance regulator gene is not associated with the clinical manifestations of cystic fibrosis. *J Clin Invest* 1992;90:785–90.
- Clancy JP, Jain M. Personalized medicine in cystic fibrosis: dawning of a new era. *Am J Respir Crit Care Med* 2012;186:593–7.
- Clancy JP, Rowe SM, Accurso FJ, Aitken ML, Amin RS, Ashlock MA, 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;67:12–8.
- Clancy JP, Szczesniak RD, Ashlock MA, Ernst SE, Fan L, Hornick DB, et al. Multicenter intestinal current measurements in rectal biopsies from CF and non-CF subjects to monitor CFTR function. *PLoS One* 2013;8:e73905.
- Comet-Boyaka E, Jablonsky M, Naren AP, Jackson PL, Muccio DD, Kirk KL. Rescuing cystic fibrosis transmembrane conductance regulator (CFTR)-processing mutants by transcomplementation. *Proc Natl Acad Sci U S A* 2004;101:8221–6.
- De Boeck K, Derichs N, Fajac I, de Jonge HR, Bronsveld I, Sermet I, et al. New clinical diagnostic procedures for cystic fibrosis in Europe. *J Cyst Fibros* 2011;10(Suppl 2):S53–66.
- De Jonge HR, Ballmann M, Veeze H, Bronsveld I, Stanke F, Tummler B, et al. Ex vivo CF diagnosis by intestinal current measurements (ICM) in small aperture, circulating Ussing chambers. *J Cyst Fibros* 2004;3(Suppl 2):159–63.
- De Lisle RC, Borowitz D. The cystic fibrosis intestine. *Cold Spring Harb Perspect Med* 2013;3:a009753.
- Dekkers JF, Wiegerinck CL, de Jonge HR, Bronsveld I, Janssens HM, de Winter-de Groot KM, et al. A functional CFTR assay using primary cystic fibrosis intestinal organoids. *Nat Med* 2013;19:939–45.
- Derichs N. Targeting a genetic defect: cystic fibrosis transmembrane conductance regulator modulators in cystic fibrosis. *Eur Respir Rev* 2013;22:58–65.
- Deriy LV, Gomez EA, Zhang G, Beacham DW, Hopson JA, Gallan AJ, et al. Disease-causing mutations in the cystic fibrosis transmembrane conductance regulator determine the functional responses of alveolar macrophages. *J Biol Chem* 2009;284:35926–38.
- Dhooghe B, Noel S, Bouzin C, Behets-Wydemans G, Leal T. Correction of chloride transport and mislocalization of CFTR protein by vardenafil in the gastrointestinal tract of cystic fibrosis mice. *PLoS One* 2013;8:e77314.
- Eckford PD, Li C, Ramjeesingh M, Bear CE. 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. *J Biol Chem* 2012;287:36639–49.
- Fernandez Alanis E, Pinotti M, Dal Mas A, Balestra D, Cavallari N, Rogalska ME, et al. An exon-specific U1 small nuclear RNA (snRNA) strategy to correct splicing defects. *Hum Mol Genet* 2012;21:2389–98.
- Flume LA, Liou TG, Borowitz DS, Li H, Yen K, Ordonez CL, et al. Ivacaftor in subjects with cystic fibrosis who are homozygous for the F508del-CFTR mutation. *Chest* 2012;142:718–24.
- French PJ, Bijman J, Bot AG, Boomaars WE, Scholte BJ, de Jonge HR. Genistein activates CFTR Cl⁻ channels via a tyrosine kinase- and protein phosphatase-independent mechanism. *Am J Physiol* 1997;273:C747–53.
- Greggio C, De Franceschi F, Figueiredo-Larsen M, Gobaa S, Ranga A, Semb H, et al. Artificial three-dimensional niches deconstruct pancreas development in vitro. *Development* 2013;140:4452–62.
- Hida K, Lai SK, Suk JS, Won SY, Boyle MP, Hanes J. Common gene therapy viral vectors do not efficiently penetrate sputum from cystic fibrosis patients. *PLoS One* 2011;6:e19919.
- Huch M, Boj SF, Clevers H. Lgr5(+) liver stem cells, hepatic organoids and regenerative medicine. *Regenerative Medicine* 2013a;8:385–7.
- Huch M, Bonfanti P, Boj SF, Sato T, Loomans CJ, van de Wetering M, et al. Unlimited in vitro expansion of adult bi-potent pancreas progenitors through the Lgr5/R-spondin axis. *EMBO J* 2013b;32:2708–21.
- Huch M, Dorrell C, Boj SF, van Es JH, Li VS, van de Wetering M, et al. In vitro expansion of single Lgr5⁺ liver stem cells induced by Wnt-driven regeneration. *Nature* 2013c;494:247–50.
- Jih KY, Hwang TC. VX-770 potentiates CFTR function by promoting decoupling between the gating cycle and ATP hydrolysis cycle. *Proc Natl Acad Sci U S A* 2013;110:4404–9.
- Joung JK, Sander JD. TALENs: a widely applicable technology for targeted genome editing. *Nat Rev Mol Cell Biol* 2013;14:49–55.
- Kalid O, Mense M, Fischman S, Shitrit A, Bihler H, Ben-Zeev E, et al. Small molecule correctors of F508del-CFTR discovered by structure-based virtual screening. *J Comput Aided Mol Des* 2010;24:971–91.
- Kim Chiaw P, Huan LJ, Gagnon S, Ly D, Sweezey N, Rotin D, et al. Functional rescue of DeltaF508-CFTR by peptides designed to mimic sorting motifs. *Chem Biol* 2009;16:520–30.
- Koulov AV, LaPointe P, Lu B, Razvi A, Coppinger J, Dong MQ, et al. Biological and structural basis for Aha1 regulation of Hsp90 ATPase activity in maintaining proteostasis in the human disease cystic fibrosis. *Mol Biol Cell* 2010;21:871–84.
- Krauss RD, Bubien JK, Drumm ML, Zheng T, Peiper SC, Collins FS, et al. Transfection of wild-type CFTR into cystic fibrosis lymphocytes restores chloride conductance at G1 of the cell cycle. *EMBO J* 1992;11:875–83.
- Lee CM, Flynn R, Hollywood JA, Scallan MF, Harrison PT. Correction of the DeltaF508 mutation in the cystic fibrosis transmembrane conductance regulator gene by zinc-finger nuclease homology-directed repair. *Biores Open Access* 2012;1:99–108.
- Leier G, Bangel-Ruland N, Sobczak K, Knieper Y, Weber WM. Sildenafil acts as potentiator and corrector of CFTR but might be not suitable for the treatment of CF lung disease. *Clin Physiol Biochem* 2012;29:775–90.
- Lim CH, Bijvelds MJ, Nigg A, Schoonderwoerd K, Houtsmuller AB, de Jonge HR, et al. Cholesterol depletion and genistein as tools to promote F508delCFTR retention at the plasma membrane. *Cell Physiol Biochem* 2007;20:473–82.
- Liu X, Luo M, Zhang LN, Yan Z, Zak R, Ding W, et al. Spliceosome-mediated RNA trans-splicing with recombinant adeno-associated virus partially restores cystic fibrosis transmembrane conductance regulator function to polarized human cystic fibrosis airway epithelial cells. *Hum Gene Ther* 2005;16:1116–23.
- Liu X, Ory V, Chapman S, Yuan H, Albanese C, Kallakury B, et al. ROCK inhibitor and feeder cells induce the conditional reprogramming of epithelial cells. *Am J Pathol* 2012;180:599–607.
- Loo TW, Bartlett MC, Clarke DM. Corrector VX-809 stabilizes the first transmembrane domain of CFTR. *Biochem Pharmacol* 2013;86:612–9.
- Lubamba B, Lecourt H, Lebacq J, Lebecque P, De Jonge H, Wallemacq P, et al. Preclinical evidence that sildenafil and vardenafil activate chloride transport in cystic fibrosis. *Am J Respir Crit Care Med* 2008;177:506–15.
- Lukacs GL, Verkman AS. CFTR: folding, misfolding and correcting the DeltaF508 conformational defect. *Trends Mol Med* 2012;18:81–91.
- Mansfield SG, Clark RH, Puttaraju M, Kole J, Cohn JA, Mitchell LG, et al. 5' exon replacement and repair by spliceosome-mediated RNA trans-splicing. *RNA* 2003;9:1290–7.
- McElroy SP, Nomura T, Torrie LS, Warbrick E, Gartner U, Wood G, et al. A lack of premature termination codon read-through efficacy of PTC124 (Ataluren) in a diverse array of reporter assays. *PLoS Biol* 2013;11:e1001593.
- Mendoza JL, Schmidt A, Li Q, Nuvaga E, Barrett T, Bridges RJ, et al. Requirements for efficient correction of DeltaF508 CFTR revealed by analyses of evolved sequences. *Cell* 2012;148:164–74.
- Nissim-Rafinia M, Aviram M, Randell SH, Shushi L, Ozeri E, Chiba-Falek O, et al. Restoration of the cystic fibrosis transmembrane conductance regulator function by splicing modulation. *EMBO Rep* 2004;5:1071–7.
- Noel S, Dhooghe B, Leal T. PDE5 inhibitors as potential tools in the treatment of cystic fibrosis. *Front Pharmacol* 2012;3:167.
- Odolczyk N, Fritsch J, Norez C, Servel N, da Cunha MF, Bitam S, et al. Discovery of novel potent DeltaF508-CFTR correctors that target the nucleotide binding domain. *EMBO Mol Med* 2013;5:1484–501.
- Okuyoneda T, Lukacs GL. Fixing cystic fibrosis by correcting CFTR domain assembly. *J Cell Biol* 2012;199:199–204.
- Okuyoneda T, Veit G, Dekkers JF, Bagdany M, Soya N, Xu H, et al. Mechanism-based corrector combination restores DeltaF508-CFTR folding and function. *Nat Chem Biol* 2013;9:444–54.
- Pedemonte N, Tomati V, Sondo E, Galletta LJ. Influence of cell background on pharmacological rescue of mutant CFTR. *Am J Physiol Cell Physiol* 2010;298:C866–74.
- Pezzulo AA, Tang XX, Hoegger MJ, Alaiwa MH, Ramachandran S, Moninger TO, et al. Reduced airway surface pH impairs bacterial killing in the porcine cystic fibrosis lung. *Nature* 2012;487:109–13.
- Phuan PW, Yang B, Knapp JM, Wood AB, Lukacs GL, Kurth MJ, et al. Cyanoquinolines with independent corrector and potentiator activities restore DeltaF508-cystic fibrosis transmembrane conductance regulator chloride channel function in cystic fibrosis. *Mol Pharmacol* 2011;80:683–93.
- Pyle LC, Ehrhardt A, Mitchell LH, Fan L, Ren A, Naren AP, et al. Regulatory domain phosphorylation to distinguish the mechanistic basis underlying acute CFTR modulators. *Am J Physiol Lung Cell Mol Physiol* 2011;301:L587–97.
- Rabeh WM, Bossard F, Xu H, Okuyoneda T, Bagdany M, Mulvihill CM, et al. Correction of both NBD1 energetics and domain interface is required to restore DeltaF508 CFTR folding and function. *Cell* 2012;148:150–63.
- Ramallo AS, Beck S, Meyer M, Penque D, Cutting GR, Amaral MD. Five percent of normal cystic fibrosis transmembrane conductance regulator mRNA ameliorates the severity of pulmonary disease in cystic fibrosis. *Am J Respir Cell Mol Biol* 2002;27:619–27.
- Ren HY, Grove DE, De La Rosa O, Houck SA, Sopha P, Van Goor F, et al. VX-809 corrects folding defects in cystic fibrosis transmembrane conductance regulator protein through action on membrane-spanning domain 1. *Mol Biol Cell* 2013;24:3016–24.

- Riordan JR. CFTR function and prospects for therapy. *Annu Rev Biochem* 2008;77:701–26.
- Rock JR, Onaitis MW, Rawlins EL, Lu Y, Clark CP, Xue Y, et al. Basal cells as stem cells of the mouse trachea and human airway epithelium. *Proc Natl Acad Sci U S A* 2009;106:12771–5.
- Rogan MP, Stoltz DA, Hornick DB. Cystic fibrosis transmembrane conductance regulator intracellular processing, trafficking, and opportunities for mutation-specific treatment. *Chest* 2011;139:1480–90.
- Rowe SM, Verkman AS. Cystic fibrosis transmembrane regulator correctors and potentiators. *Cold Spring Harb Perspect Med* 2013;2013.
- Sato T, Clevers H. Growing self-organizing mini-guts from a single intestinal stem cell: mechanism and applications. *Science* 2013;340:1190–4.
- Sato T, Stange DE, Ferrante M, Vries RG, Van Es JH, Van den Brink S, et al. Long-term expansion of epithelial organoids from human colon, adenoma, adenocarcinoma, and Barrett's epithelium. *Gastroenterology* 2011;141:1762–72.
- Sato T, Vries RG, Snippert HJ, van de Wetering M, Barker N, Stange DE, et al. Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. *Nature* 2009;459:262–5.
- Schwank G, Koo BK, Sasselli V, Dekkers JF, Heo I, Demircan T, et al. Functional repair of CFTR by CRISPR/Cas9 in intestinal stem cell organoids of cystic fibrosis patients. *Cell Stem Cell* 2013;13:653–8.
- Sermet-Gaudelus I, Boeck KD, Casimir GJ, Vermeulen F, Leal T, Mogenet A, et al. Ataluren (PTC124) induces cystic fibrosis transmembrane conductance regulator protein expression and activity in children with nonsense mutation cystic fibrosis. *Am J Respir Crit Care Med* 2010;182:1262–72.
- Sohma Y, Yu YC, Hwang TC. Curcumin and genistein: the combined effects on disease-associated CFTR mutants and their clinical implications. *Curr Pharm Des* 2013;19:3521–8.
- Sorio C, Buffelli M, Angiari C, Ettore M, Johansson J, Vezzalini M, et al. Defective CFTR expression and function are detectable in blood monocytes: development of a new blood test for cystic fibrosis. *PLoS One* 2011;6:e22212.
- Suprynowicz FA, Upadhyay G, Krawczyk E, Kramer SC, Hebert JD, Liu X, et al. Conditionally reprogrammed cells represent a stem-like state of adult epithelial cells. *Proc Natl Acad Sci U S A* 2012;109:20035–40.
- Tamanini A, Borgatti M, Finotti A, Piccagli L, Bezzetti V, Favia M, et al. Trimethylangelicin reduces IL-8 transcription and potentiates CFTR function. *Am J Physiol Lung Cell Mol Physiol* 2011;300:L380–90.
- Tang XX, Fok KL, Chen H, Chan KS, Tsang LL, Rowlands DK, et al. Lymphocyte CFTR promotes epithelial bicarbonate secretion for bacterial killing. *J Cell Physiol* 2012;227:3887–94.
- Van de Weert-van Leeuwen PB, Van Meegen MA, Speirs JJ, Pals DJ, Rooijackers SH, Van der Ent CK, et al. Optimal complement-mediated phagocytosis of *Pseudomonas aeruginosa* by monocytes is cystic fibrosis transmembrane conductance regulator-dependent. *Am J Respir Cell Mol Biol* 2013;49:463–70.
- Van Goor F, Hadida S, Grootenhuys PD, Burton B, Stack JH, Straley KS, 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;108:18843–8.
- Van Goor F, Yu H, Burton B, Hoffman BJ. Effect of ivacaftor on CFTR forms with missense mutations associated with defects in protein processing or function. *J Cyst Fibros* 2014;13:29–36.
- Wang W, Bernard K, Li G, Kirk KL. Curcumin opens cystic fibrosis transmembrane conductance regulator channels by a novel mechanism that requires neither ATP binding nor dimerization of the nucleotide-binding domains. *J Biol Chem* 2007;282:4533–44.
- Wilschanski M. Novel therapeutic approaches for cystic fibrosis. *Discov Med* 2013;15:127–33.
- Wilschanski M, Miller LL, Shoseyov D, Blau H, Rivlin J, Aviram M, et al. Chronic ataluren (PTC124) treatment of nonsense mutation cystic fibrosis. *Eur Respir J* 2011;38:59–69.
- Wolde M, Fellows A, Cheng J, Kivenson A, Coutermarsh B, Talebian L, et al. Targeting CAL as a negative regulator of DeltaF508-CFTR cell-surface expression: an RNA interference and structure-based mutagenetic approach. *J Biol Chem* 2007;282:8099–109.
- Wong AP, Bear CE, Chin S, Pasceri P, Thompson TO, Huan LJ, et al. Directed differentiation of human pluripotent stem cells into mature airway epithelia expressing functional CFTR protein. *Nat Biotechnol* 2012;30:876–82.
- Xue X, Mutyam V, Tang L, Biswas S, Du M, Jackson LA, et al. Synthetic aminoglycosides efficiently suppress CFTR nonsense mutations and are enhanced by ivacaftor. *Am J Respir Cell Mol Biol* 2013.
- Yu H, Burton B, Huang CJ, Worley J, Cao D, Johnson JP Jr, et al. Ivacaftor potentiation of multiple CFTR channels with gating mutations. *J Cyst Fibros* 2012;11:237–45.
- Yu YC, Miki H, Nakamura Y, Hanyuda A, Matsuzaki Y, Abe Y, et al. Curcumin and genistein additively potentiate G551D-CFTR. *J Cyst Fibros* 2011;10:243–52.
- Yui S, Nakamura T, Sato T, Nemoto Y, Mizutani T, Zheng X, et al. Functional engraftment of colon epithelium expanded in vitro from a single adult Lgr5(+) stem cell. *Nat Med* 2012;18:618–23.
- Zamecnik PC, Raychowdhury MK, Tabatadze DR, Cantiello HF. Reversal of cystic fibrosis phenotype in a cultured Delta508 cystic fibrosis transmembrane conductance regulator cell line by oligonucleotide insertion. *Proc Natl Acad Sci U S A* 2004;101:8150–5.