Organoid-guided synergistic treatment of minimal function CFTR mutations with CFTR modulators, roflumilast and simvastatin: a personalised approach

Received: 17 May 2023
Accepted: 23 Sept 2023

To the Editor:

Highly effective cystic fibrosis transmembrane conductance regulator (CFTR) protein-targeting modulator therapies (HEMTs) facilitate strong clinical improvements in a large proportion of people with cystic fibrosis (CF) [1, 2]. More specifically, the European Medicines Agency and US Food and Drug Administration (FDA) approved combination of the CFTR modulators elexacaftor/tezacaftor/ivacaftor (ETI) for people with CF with at least one F508del allele, while the FDA extended eligibility for several rare genotypes [3, 4]. However, 10–15% of those with CF carry CFTR mutations that are unresponsive to HEMTs as monotherapy [1]; furthermore, some suffer from HEMT intolerance, and HEMTs are sometimes not accessible due to practical challenges, such as lack of access due to high costs or legislation and approval challenges. Consequently, the focus in the CF research field has shifted towards filling the unmet clinical need for the people with CF that will not benefit from HEMTs.

We previously carried out large drug repurposing screens using patient-derived intestinal organoids (PDIOs) from CF patients with rare CFTR variants using forskolin-induced swelling (FIS) assays, allowing characterisation of functional CFTR [5, 6]. FIS outcomes associate with clinical features of CF and treatment response, enabling compound testing in a personalised setting [3]. Three FDA-approved compound families, with favourable safety and pharmacokinetic profiles, were found to increase CFTR function. We established that CFTR modulators have a large treatment potential for CFTR mutations that are not eligible for these compounds at present [6]. We characterised two additional FDA-approved compound families that were not previously described to increase CFTR function. Phosphodiesterase 4 (PDE4) inhibitors, such as roflumilast, which is approved for obstructive lung disease treatment, presumably increase CFTR function by elevating intracellular cAMP levels, thereby increasing opening of available CFTR [6]. We additionally identified statins, such as simvastatin, to increase CFTR function in the context of W1282X/W1282X CFTR, when combined with CFTR modulator pretreatment [5]. The molecular mechanism connecting statin treatment to increased W1282X CFTR function remains unclear and previous clinical studies of statin monotherapy did not show effects in people with CF (NCT00255242) [5].

Due to the diverse molecular working mechanisms of ETI, roflumilast and statins, the combination could synergistically restore CFTR function. Here, we tested this hypothesis in PDIOs using the FIS assay for two different CFTR genotypes that are not presently eligible for ETI and have previously been characterised as unresponsive to ETI monotreatment: L927P/W1282X and W1282X/W1282X [4–8]. L927P is a rare missense mutation (c.2780C>T; pLeu927Pro; allele frequency of 0.02% in CFTR2) that is overrepresented in the Belgian CF population with a 2.4% incidence rate [9, 10]. L927P is a complex allele, due to the presence of an additional 1110delGAAT mutation in cis which impacts splicing and thereby potentially masks response to CFTR modulators [8]. W1282X is the most prevalent premature termination codon (PTC) mutation after G542X (c.3846G>A; p.Trp128X; 1.2% allele frequency in CFTR2) with an incidence of 61% in Jewish Ashkenazi people with CF [11, 12]. PTC mutations result in low levels of truncated CFTR protein and associate with severe clinical manifestations [13]. The main preclinical treatment strategy for PTC mutations is inducing translational readthrough at the PTC, yet in clinical settings, results of such readthrough compounds are disappointing [14].

Shareable abstract (@ERSpublications)
This study describes how preclinical research has guided a successful personalised clinical treatment regimen in a person with minimal function CFTR, upon a synergistic treatment regimen consisting of CFTR modulators, simvastatin and roflumilast https://bit.ly/3rDTHZL

Cite this article as: Spelier S, de Winter-de Groot K, Keijzer-Nieuwenhuijze N, *et al*. Organoid-guided synergistic treatment of minimal function CFTR mutations with CFTR modulators, roflumilast and simvastatin: a personalised approach. *Eur Respir J* 2024; 63: 2300770 [DOI: 10.1183/13993003.00770-2023].

Here, we describe preclinical data of the drug combination of ETI, roflumilast and simvastatin in PDIOs and present a case study where an individual with L927P/W1272X CFTR with a severe, deteriorating clinical status received this drug combination under a compassionate use setting covered by the patient's own hospital (figure 1a).

CFTR restoration was studied in PDIOs of an individual with W1282X/L927P CFTR and four W1282X/W1282X PDIOs. All PDIOs showed no baseline residual CFTR function. Compounds used as monotherapy or in dual combinations were mostly ineffective, yet the complete drug combination resulted in significant FIS for both genotypes, which is most clear in 3-h measurements (figure 1b and c). Individual variation was observed between the four W1282X/W1282X PDIOs, yet trends across the various drug combinations were identical. Rescue of CFTR with the complete compound combination was higher than lumacaftor/ivacaftor FIS in F508del/minimal function (MF) for both genotypes, similar to lumacaftor/ivacaftor in F508del/F508del PDIOs and reached approximately 40% of the effect of ETI in F508del/MF PDIOs.

As the patient with L927P/W1282X CFTR experienced rapid clinical deterioration, we initiated treatment with the complete drug combination in a clinical N-of-1 setting. The 51-year-old male patient colonised with a multi-resistant *Pseudomonas aeruginosa* received an average of four annual antipseudomonal antibiotics per year for exacerbations and received no previous CFTR modulators. Other major CF comorbidities were CF-related diabetes, pancreatic insufficiency and polyposis nasi. In recent years, his rapidly deteriorating clinical condition resulted in permanent incapacitation for work.

A trial period with simvastatin (40 mg daily), roflumilast (250 µg daily) and ETI treatment was started. Dosages were selected based on labelled indications for use in people without CF, and together with a pharmacist a drug safety monitoring plan was designed [3, 15, 16]. Baseline measurements of the patient were a forced expiratory volume in 1 s (FEV_1) of 74% predicted, corresponding to an absolute FEV_1 of 2.52 L (day 0 of therapy start). During evaluation with his pulmonologist a week after treatment, the patient reported a major decrease of productive cough and fatigue. After 4 weeks, sweat chloride (SwCl) concentrations lowered from 106 mM to 58 mM and decrease persisted at 16 weeks of treatment (67 mM; −39 mM compared to baseline) (figure 1d). Pulmonary function measurements improved considerably after 16 weeks, underlined by an absolute FEV_1 of 2.85 L, corresponding to an absolute increase of 9% (figure 1e). The Cystic Fibrosis Questionnaire-Revised (CFQ-R) respiratory domain improved after 4 weeks treatment by 61 points (minimal clinically important difference (MCID) 4 points) and 67 points in the vitality domain on the 100 point scale (no MCID available), and also on other subdomains (figure 1f) [17, 18]. The self-report depression screening (PHQ-9) and anxiety questionnaires improved considerably as well (figure 1f). Importantly, over the course of the treatment, no adverse effects were detected and drug safety indicators, such as liver function, showed no anomalies.

Here, we report that a personalised drug testing effort led to a successful clinical intervention with a novel drug combination consisting of the FDA-approved compounds, ETI, roflumilast and simvastatin, in a person with two minimal function CFTR mutations. The potential for the individual drugs to increase CFTR function was based on previous observations, but the novel combinatory regimen was verified here using PDIOs prior to clinical intervention.

In PDIOs, FIS data highlighted that the drugs interact in a synergistic manner. Whilst single drugs were not effective, restoration of CFTR function with the complete compound combination was similar to lumacaftor/ivacaftor-induced FIS in homozygous F508del PDIOs. This points to the direction of its clinical potential as lumacaftor/ivacaftor yields moderate results in homozygous F508del people with CF in a clinical setting as well [19]. The observed synergy between the compounds is likely due to the different molecular mechanisms of the compounds. ETI directly interacts with the produced CFTR protein leading to improved folding, trafficking and gating of CFTR protein, and PDE4 inhibitors such as roflumilast can result in increased activation of CFTR by elevating intracellular cAMP levels. The exact working mechanism of simvastatin in terms of restoring CFTR function is unclear and future studies on the exact mechanism of action of statins are warranted. FIS effects were clearer when measuring swelling over a 3-h period. Whilst previous studies established optimal *in vitro–in vivo* correlations with 1-h measurements, 3-h measurements can enable better signal-over-noise measurements for *in vitro–in vivo* correlations if FIS at 1-h measurement is low [20].

Clinically, the magnitude of response is exciting and strongly supports individual clinical benefit, but additional studies are needed. SwCl decrease was similar to observations in F508del/MF ETI-treated people with CF, suggesting drug-induced effects well beyond individual technical variation [21, 22].

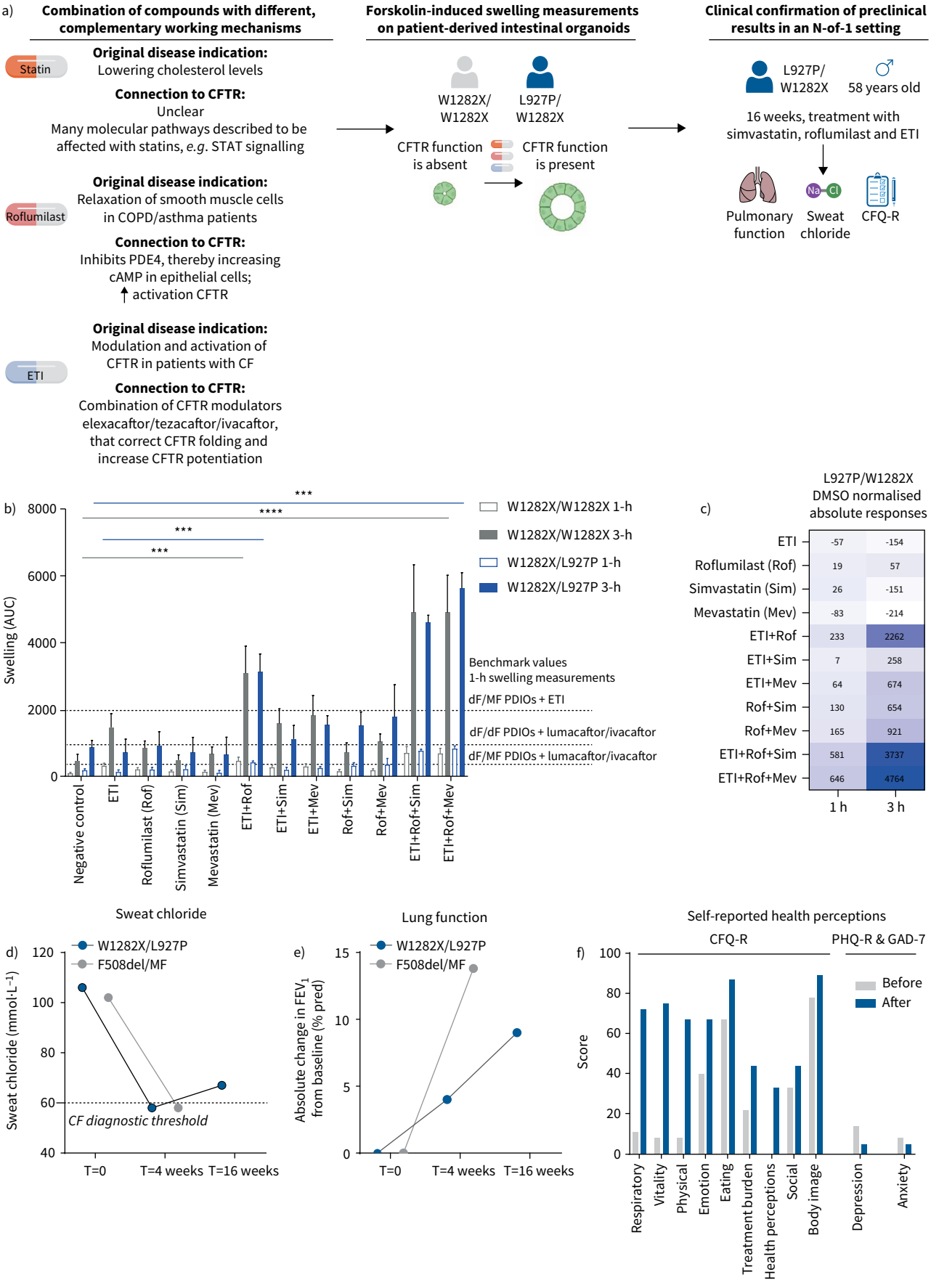
a)
Combination of compounds with different, complementary working mechanisms
Forskolin-induced swelling measurements on patient-derived intestinal organoids
Clinical confirmation of preclinical results in an N-of-1 setting
Statin
Original disease indication:
Lowering cholesterol levels
Connection to CFTR:
Unclear
Many molecular pathways described to be affected with statins, e.g. STAT signalling
Roflumilast
Original disease indication:
Relaxation of smooth muscle cells in COPD/asthma patients
Connection to CFTR:
Inhibits PDE4, thereby increasing cAMP in epithelial cells;
↑ activation CFTR
ETI
Original disease indication:
Modulation and activation of CFTR in patients with CF
Connection to CFTR:
Combination of CFTR modulators elexacaftor/tezacaftor/ivacaftor, that correct CFTR folding and increase CFTR potentiation
W1282X/ W1282X
L927P/ W1282X
CFTR function is absent
CFTR function is present
L927P/ W1282X
58 years old
16 weeks, treatment with simvastatin, roflumilast and ETI
Pulmonary function
Sweat chloride
CFQ-R
b)
Swelling (AUC)
W1282X/W1282X 1-h
W1282X/W1282X 3-h
W1282X/L927P 1-h
W1282X/L927P 3-h
Benchmark values
1-h swelling measurements
dF/MF PDIOs + ETI
dF/dF PDIOs + lumacaftor/ivacaftor
dF/MF PDIOs + lumacaftor/ivacaftor
Negative control
ETI
Roflumilast (Rof)
Simvastatin (Sim)
Mevastatin (Mev)
ETI+Rof
ETI+Sim
ETI+Mev
Rof+Sim
Rof+Mev
ETI+Rof+Sim
ETI+Rof+Mev
c)
L927P/W1282X
DMSO normalised
absolute responses
ETI -57 -154
Roflumilast (Rof) 19 57
Simvastatin (Sim) 26 -151
Mevastatin (Mev) -83 -214
ETI+Rof 233 2262
ETI+Sim 7 258
ETI+Mev 64 674
Rof+Sim 130 654
Rof+Mev 165 921
ETI+Rof+Sim 581 3737
ETI+Rof+Mev 646 4764
1 h
3 h
Sweat chloride
d)
W1282X/L927P
F508del/MF
Sweat chloride (mmol·L⁻¹)
CF diagnostic threshold
T=0
T=4 weeks
T=16 weeks
Lung function
e)
W1282X/L927P
F508del/MF
Absolute change in FEV1 from baseline (% pred)
T=0
T=4 weeks
T=16 weeks
Self-reported health perceptions
f)
CFQ-R
PHQ-R & GAD-7
Before
After
Score
Respiratory
Vitality
Physical
Emotion
Eating
Treatment burden
Health perceptions
Social
Body image
Depression
Anxiety

FIGURE 1 Preclinical studies guide a successful personalised clinical treatment regimen in a person with minimal function cystic fibrosis transmembrane conductance regulator (CFTR), upon synergistic treatment with CFTR modulators, a cholesterol synthase inhibitor and a phosphodiesterase 4 (PDE4) inhibitor. a) Schematic pipeline of this study. We characterised synergetic effect of statins, roflumilast and elexacaftor/tezacaftor/ivacaftor (ETI) in a preclinical setting on patient-derived intestinal organoids (PDIOs) using the functional forskolin-induced swelling (FIS) assay, after which we characterised efficacy of simvastatin/roflumilast/ETI in a clinical N-of-1 setting in the patient with L927P/W1282X CFTR. b) CFTR function characterised by FIS in PDIOs with genotypes W1282X/L927P (indicated in blue) or W1282X/W1282X (indicated in grey), measured during 1 h or 3 h. Different compound combinations are indicated on the *x*-axis. Bars indicate the average of three biological replicates based on three technical replicates with error bars indicating the standard error of the mean. Significance was calculated using a two-way ANOVA followed by Dunnett's multiple comparisons test. ***: p<0.001; ****: p<0.0001. Dotted lines indicate benchmark 1-hour FIS values for F508del (dF)/minimal function (MF) or dF/dF PDIOs treated with indicated CFTR modulators. AUC: area under the curve. c) Absolute responses normalised for dimethyl sulfoxide (DMSO) response for L927P/W1282X PDIOs. d) Sweat chloride concentration at day 0 of treatment and after 4 or 16 weeks of treatment in the N-of-1 trial of a cystic fibrosis (CF) patient with L927P/W1282X CFTR, compared to data of F508del/MF CF patients as published by Middleton *et al*. [22]. The dotted line indicates the CF diagnostic threshold. e) Overview of lung function of the treated patient at day 0 of treatment and after 4 or 16 weeks upon treatment with ETI/roflumilast/simvastatin, compared to data of F508del/MF CF patients as published by Middleton *et al*. [22]. Lung function is depicted as absolute change in forced expiratory volume in 1 s (FEV_1) % predicted. f) Overview of self-reported health perceptions before (grey bars) and 4 weeks after treatment (blue bars), based on Cystic Fibrosis Questionnaire-Revised (CFQ-R) domain score (range 0 to 100; higher scores indicate a higher patient-reported quality of life with regard to the concerning domain), Personal Health Questionnaire (PHQ)-9 depression score (range 0 to 27; higher scores indicating a depressive disorder) and Generalized Anxiety Disorder (GAD)-7 questionnaire (range 0 to 21; higher scores indicating a generalised anxiety disorder).

FEV_1 % predicted reaches two-thirds of the increase as measured in F508del/MF ETI-treated people with CF, yet in absolute numbers the 9% increase in FEV_1 is substantial and beyond the 5% threshold that is considered clinically relevant [22]. The major improvements in self-reported health perceptions furthermore underline major health improvements for the patient. While it could be argued that the increase in FEV_1 is partially due to roflumilast-mediated smooth muscle relaxation and, as such, bronchovasodilation, the change in SwCl concentration and the preclinical PDIO data support direct modulation of CFTR *in vivo*. Although the magnitude of response is encouraging for this individual, a washout period showing subsequent decline is missing. As such, this single case observation needs to be interpreted with care and points to a need for additional studies. Future studies in larger subgroups with placebo-controlled cohorts should investigate the contribution of the different drugs in the context of different genotypes, such as homozygous W1282X CFTR.

Overall, our study shows that personalised drug testing effort can result in identification of novel treatment regimens and successful individual clinical intervention. Proceeding with further clinical studies on the combination of ETI, roflumilast and simvastatin is essential to identify how more people with CF can benefit from this therapeutic regimen.

Sacha Spelier[1,2], Karin de Winter-de Groot[1], Natascha Keijzer-Nieuwenhuijze[1,2], Yves Liem[3], Kors van der Ent[1], Jeffrey Beekman[1,2,5] and Lieke S. Kamphuis[4,5]

[1]Department of Pediatric Respiratory Medicine, Wilhelmina Children's Hospital, University Medical Center, Utrecht University, Utrecht, The Netherlands. [2]Regenerative Medicine Utrecht, University Medical Center, Utrecht University, Utrecht, The Netherlands. [3]Department of Clinical Pharmacy, Wilhelmina Children's Hospital, University Medical Center, Utrecht University, Utrecht, The Netherlands. [4]Department of Respiratory Medicine, Erasmus University Medical Center, Rotterdam, The Netherlands. [5]J. Beekman and L.S. Kamphuis contributed equally to this article as lead authors and supervised the work.

Corresponding author: Lieke S. Kamphuis (l.kamphuis@erasmusmc.nl)

Ethics statement: All experimentation using human tissues described herein was approved by the medical ethical committee at University Medical Center Utrecht (UMCU; TcBio#19-831). Informed consent for intestinal tissue collection, generation, storage and use of the organoids was obtained from all participating patients.

Conflict of interest: J. Beekman and K. van der Ent are inventors on patent(s) related to organoid swelling, and received royalties from 2017 onwards. L.S. Kamphuis received royalties from Vertex for participation in the advisory board for psychosocial wellbeing in CF patients in 2022. All other authors declare no competing interests.

Support statement: This work was supported by Emily's Entourage (grant: R1022). Funding information for this article has been deposited with the Crossref Funder Registry.

References

1 McBennett KA, Davis PB, Konstan MW. Increasing life expectancy in cystic fibrosis: advances and challenges. *Pediatr Pulmonol* 2022; 57: Suppl. 1, S5–S12.
2 Riordan JR, Rommens JM, Kerem B, *et al.* Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. *Science* 1989; 245: 1066–1073.
3 European Medicines Agency. Kaftrio EMA Approval. 2023. www.ema.europa.eu/en/medicines/human/EPAR/kaftrio#assessment-history-section
4 US Food and Drug Administration. Kaftrio FDA Approval. 2023. www.accessdata.fda.gov/scripts/cder/daf/index.cfm?event=overview.process&ApplNo=212273
5 Spelier S, de Poel E, Ithakisiou GN, *et al.* High-throughput functional assay in cystic fibrosis patient-derived organoids allows drug repurposing. *ERJ Open Res* 2023; 9: 00495-2022.
6 de Poel E, Spelier S, Hagemeijer MC, *et al.* FDA-approved drug screening in patient-derived organoids demonstrates potential of drug repurposing for rare cystic fibrosis genotypes. *J Cyst Fibros* 2023; 22: 548–559.
7 Burgel P-R, Sermet-Gaudelus I, Durieu I, *et al.* The French compassionate programme of elexacaftor/tezacaftor/ivacaftor in people with cystic fibrosis with advanced lung disease and no F508del *CFTR* variant. *Eur Respir J* 2023; 61: 2202437.
8 Ramalho AS, Cuyx S, Boon M, *et al.* The importance of CFTR mRNA testing to uncover other variants in CF genotype that affect CFTR expression. https://researchportal.vub.be/en/publications/the-importance-of-cftr-mrna-testing-to-uncover-other-variants-in-
9 Levring J, Terry DS, Kilic Z, *et al.* CFTR function, pathology and pharmacology at single-molecule resolution. *Nature* 2023; 616: 606–614.
10 Storm K, Moens E, Vits L, *et al.* High incidence of the CFTR mutations 3272-26A→G and L927P in Belgian cystic fibrosis patients, and identification of three new CFTR mutations (186-2A→G, E588V, and 1671insTATCA). *J Cyst Fibros* 2007; 6: 371–375.
11 Kalman YM, Kerem E, Darvasi A, *et al.* Difference in frequencies of the cystic fibrosis alleles, ΔF508 and W1282X, between carriers and patients. *Eur J Hum Genet* 1994; 2: 77–82.
12 Shoshani T, Augarten A, Gazit E, *et al.* Association of a nonsense mutation (W1282X), the most common mutation in the Ashkenazi Jewish cystic fibrosis patients in Israel, with presentation of severe disease. *Am J Hum Genet* 1992; 50: 222–228.
13 de Poel E, Spelier S, Suen SWF, *et al.* Functional restoration of CFTR nonsense mutations in intestinal organoids. *J Cyst Fibros* 2022; 21: 246–253.
14 Spelier S, van Doorn EPM, van der Ent CK, *et al.* Readthrough compounds for nonsense mutations: bridging the translational gap. *Trends Mol Med* 2023; 29: 297–314.
15 European Medicines Agency. Daxas EMA Approval. 2023. www.ema.europa.eu/en/medicines/human/EPAR/daxas
16 European Medicines Agency. Simvastatin EMA Approval. 2023. www.ema.europa.eu/en/medicines/human/referrals/simvastatin-vale
17 Quittner AL, Modi AC, Wainwright C, *et al.* Determination of the minimal clinically important difference scores for the Cystic Fibrosis Questionnaire-Revised respiratory symptom scale in two populations of patients with cystic fibrosis and chronic *Pseudomonas aeruginosa* airway infection. *Chest* 2009; 135: 1610–1618.
18 Sutharsan S, Mondéjar-Lopez P, Duckers J, *et al.* P117 A longitudinal study on the impact of elexacaftor/tezacaftor/ivacaftor treatment on quality of life in people with cystic fibrosis in the real world. *J Cyst Fibros* 2023; 22: Suppl. 2, S99–S100.
19 Muilwijk D, Bierlaagh M, van Mourik P, *et al.* Prediction of real-world long-term outcomes of people with CF homozygous for the F508del mutation treated with CFTR modulators. *J Pers Med* 2021; 11: 1376.
20 de Winter-de Groot KM, Janssens HM, van Uum RT, *et al.* Stratifying infants with cystic fibrosis for disease severity using intestinal organoid swelling as a biomarker of CFTR function. *Eur Respir J* 2018; 52: 1702529.
21 Vermeulen F, Lebecque P, De Boeck K, *et al.* Biological variability of the sweat chloride in diagnostic sweat tests: a retrospective analysis. *J Cyst Fibros* 2017; 16: 30–35.
22 Middleton PG, Mall MA, Dřevínek P, *et al.* Elexacaftor–tezacaftor–ivacaftor for cystic fibrosis with a single Phe508del allele. *N Engl J Med* 2019; 381: 1809–1819.