Personalized medicine with drugs targeting the underlying protein defect in cystic fibrosis: is monitoring of treatment response necessary?

Katharina Niedermayr, Verena Gasser, Claudia Rueckes-Nilges, Dorothea Appelt, Johannes Eder, Teresa Fuchs, Lutz Naehrlich and Helmut Ellemunter

Abstract: Cystic fibrosis (CF) is caused by two mutations in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene. In the last years, drugs targeting the underlying protein defect like lumacaftor/ivacaftor (LUM/IVA) or tezacaftor/ivacaftor (TEZ/IVA) and more recently elexacaftor/tezacaftor/ivacaftor (ELX/TEZ/IVA) were admitted. Outcome parameters evaluating therapy response like forced expiratory pressure in 1 s (FEV₁), body mass index (BMI) or the efficacy of CFTR function in sweat glands showed improvement in several cases. Other, CFTR biomarkers were analysed rarely. This prospective observational study was aimed at evaluating CFTR function in patients treated with different CFTR modulators together with common valid clinical outcome parameters at standardized appointments (day 0, week 2, 4, 16). We followed four patients with the same mutation (F508del-CFTR), sex, age and disease severity. Monitoring focused on lung function, gastrointestinal aspects and CFTR function of sweat glands, nasal and intestinal epithelium. Sweat tests were performed by pilocarpine iontophoresis. Nasal potential difference (NPD) measured transepithelial voltage in vivo and potential increased when CFTR function improved. Rectal biopsies were obtained for intestinal current measurements (ICM) ex vivo. Intestinal CFTR function was assessed by stimulating chloride secretion with different reagents. Response to CFTR modulators regarding clinical outcome parameters was rather variable. A sweat chloride reduction of 35.3 mmol/L, nasal CFTR rescue of 4.4% and fivefold higher CFTR function in the intestine was seen at week 16 post-LUM/IVA. Due to our monitoring, we identified a non-responder to LUM/IVA and TEZ/IVA. In case of ELX/TEZ/IVA, clinical parameters and CFTR bioassays improved and were concordant. Although our cohort is small, results emphasize that non-responders exist and conclusions could not be drawn if patients were not monitored. Data on CFTR function can confirm or disprove ongoing CFTR dysfunction and might be helpful selectively. Non-responders need other alternative therapy options as demonstrated with ELX/TEZ/IVA.

Keywords: CFTR modulator therapy, cystic fibrosis, drug reactions, intestinal current measurements, nasal potential difference

Received: 17 January 2022; revised manuscript accepted: 1 June 2022.

Introduction
In Caucasians, cystic fibrosis (CF) is the most common life-threatening genetic disease. Nevertheless, CF is defined as a ‘rare disease’. CF is caused by two mutations in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene on the long arm of chromosome 7. CFTR is a chloride channel, which is activated through a cyclic adenosine monophosphate (cAMP) depending protein kinase A. In detail, CFTR, an
ATP-binding cassette transporter C member, comprises two membrane-spanning domains (MSDs), two nucleotide-binding domains (NBDs), and a regulatory domain. It plays an important role in the epithelial ion and water household on the surface of mucous membranes of different organs. **CFTR** protein is not working correctly due to ‘loss of number’ and / or ‘loss of function’ mutations (e.g. absence, dysfunction). Until now, there are over 2100 different mutations identified and more than 350 are known to cause disease. The most common mutation in patients with CF is the F508del mutation. The prevalence of F508del mutation varies between countries, for example, in the Italian population F508del occurs less often than in the Northern European population and ethnical differences can be found too. F508del belongs to class II mutations. The amino acid phenylalanine on position 508 in the **CFTR** protein is missing. Therefore, the **CFTR** protein is misfolded in the endoplasmic reticulum and is not passed to the membrane surface. As a result, **CFTR** cannot work correctly and will be removed by the proteasome.

New therapy approaches focus on this underlying protein defect and led to the admission of mutation specific **CFTR** correctors or potentiators like lumacaftor/ivacaftor (LUM/IVA) or tezacaftor/ivacaftor (TEZ/IVA) and more recently with elexacaftor/tezacaftor/ivacaftor (ELX/TEZ/IVA).

**CFTR** function can be evaluated by several **CFTR** bioassays in **vitro** and **ex vivo**. The most common one is the sweat test, because according to guidelines CF is diagnosed by elevated sweat chloride. Sweat is obtained by pilocarpine iontophoresis and concentration of electrolytes can be measured. A high extracellular concentration of sodium and chloride due to decreased reabsorption of NaCl in patients with a **CFTR** defect can be found. A chloride sweat level of \( \leq 29 \text{ mmol/L} \) is found in healthy individuals, whereas patients with CF have levels \( \geq 60 \text{ mmol/L} \). Furthermore, other **CFTR** bioassays should be considered to clarify the diagnosis in patients with inconclusive sweat test. **CFTR** bioassays like nasal potential difference (NPD) or intestinal current measurements (ICM) can be used for confirmation of **CFTR** dysfunction. However, these bioassays are more difficult to perform than other electrophysiological measurements (e.g. sweat test). It requires extensive training and specialized equipment listed in the Standard Operating Procedures (SOP) of the European Cystic Fibrosis Society (ECFS) Diagnostic Network Working Group and Clinical Trials Network. NPD measures the transepithelial potential difference between the Regio respiratoria (inferior nasal turbinate) and a subcutaneous electrode placed at the forearm. In patients with CF a more negative basal potential difference (PD) due to a defective chloride secretion and a hyperabsorption of sodium can be measured. During the measurement, the sodium channel and afterwards the chloride channel will be blocked. A decreased potential difference is pathognomonic for this disease and the function of the chloride channel can be predicted. The same current measurements as discussed before can be performed on the intestinal epithelium (ICM) ex vivo. After stimulation of **CFTR**-mediated chloride secretion in ex vivo rectal biopsies, the circuit current \( (I_c) \) will be measured as a value of ion transport. In patients with CF only a small amount of chloride will be detected; in healthy individuals, it will be a huge current.

Our observation was aimed at evaluating the effect of different **CFTR** modulators on **CFTR** assembly via sweat test, nasal potential difference (NPD) and intestinal current measurements (ICM) as well as common valid clinical outcome parameters (FEV\(_1\), LCI\(_{2.5}\%), \text{BMI} \) in real-time setting.

**Subjects**

**Patient 1** (female, aged early 20s, homozygous F508del-**CFTR** mutation) with severe CF lung disease at therapy start, experienced several pulmonary exacerbations resulting in restricted lung function, ventilation inhomogeneity of small airways and visualized severe bronchiectasis as well as mucous plugging in multidetector computed tomography (MDCT) scans. At the beginning of our study the patient’s body mass index (BMI) was low and the patient was pancreatic insufficient (faecal elastase <16µg/g) with an elevated faecal calprotectin. Sweat chloride concentration was 100 mmol/L (CF range \( \geq 60 \text{ mmol/L} \)). At day 0 the **CFTR** diagnostic nasal epithelium (NPD) measurements revealed a mean recovery of 9.42 mV (CF range >-8 mV), resulted in a Wilschanski score of 1.55 (CF range >0.70) and in a Sermet score of -2.11 (CF range <0.27) indicating CF. A low short circuit current due to a moderate increase of potential...
difference by adding stimulators like forskolin/3-Isobutyl-1-methylxanthine (IBMX), carbachol or histamine ($\Delta I_{sc}$ forsk/IBMX, carba + hist 5.88 and 24.30 $\mu$A/cm$^2$, respectively, for the best biopsy) was obtained with ICM as characteristic for CF patients (cut-off <39 $\mu$A/cm$^2$).

According to her CFTR function in sweat glands, nasal and intestinal epithelium patient 1 was improving with the combination therapy LUM/IVA. Furthermore, an improvement was seen in lung function parameters at week 16. No improvement concerning LCI$_{2.5\%}$ or BMI could be found. Slightly lower faecal calprotectin was found after LUM/IVA initiation. Similarly, to the increased faecal calprotectin, C-reactive protein was elevated [0.77–1.84 mg/dl (reference value <0.5 mg/dl)] constantly throughout observational period. Medication response until 16 weeks after first intake of LUM/IVA can be seen in Tables 1 and 2.

Three years after first dose of LUM/IVA a stabilization in values for lung function and BMI was detected, while CFTR function was worse. LUM/IVA was stopped, and ELX/TEZ/IVA was started following the same prospective observational investigations (Table 2).

**Patient 2** (female, aged early 20s, homozygous F508del-CFTR mutation) had a severe lung disease and moderate bronchiectasis as well as mucous plugging in MDCT scans. Patient 2 was classified as underweight with a BMI of 18.3 kg/m$^2$ and showed pancreatic insufficiency (stool elastase <16 $\mu$g/g). Sweat chloride was 105 mmol/L/104 mmol/L. NPD measurement achieved mean recovery of -6.55 mV, Wilschanski score 0.88 and Sermet score -1.79. Furthermore, ICM showed a low $\Delta I_{sc}$ forsk/IBMX, carba + hist (6.84 and 26.56 $\mu$A/cm$^2$, respectively, for the best biopsy) due to slight cAMP activation and only little cholinergic CFTR chloride secretion.

These findings were comparable to patient 1. But patient 2 did not show a response to the combination therapy with LUM/IVA relating to lung function parameters, BMI, sweat test, NPD. Only a slightly lower LCI$_{2.5\%}$ regarding fewer ventilation inhomogeneity and a higher, but not satisfying intestinal CFTR function could be obtained at week 16 compared with day 0. So, due to a lack of improvement in several clinical outcome parameters at week 16 (Tables 1 and 3), patient 2 was categorized as non-responder for LUM/IVA. The combination therapy of LUM/IVA was finally stopped after 1 year of observation and TEZ/IVA was started. The same monitoring for TEZ/IVA was performed (Table 3) but did not lead to acceptable response and non-responder status for TEZ/IVA was verified again. Recently, patient 2 started the intake of ELX/TEZ/IVA and is improving under the combination therapy respecting nearly all evaluated outcome parameters (Table 3).

**Patient 3** (female, aged early 20s, homozygous F508del-CFTR mutation) with moderate CF lung disease and pancreas insufficiency at therapy start with LUM/IVA. Faecal calprotectin was not elevated. Sweat chloride concentration, NPD and ICM scores indicated CF. Patient 3 was improving under the combination therapy with LUM/IVA regarding all clinical outcome parameters (e.g. lung function parameters, LCI$_{2.5\%}$ and BMI). Furthermore, CFTR function in sweat glands as well as in nasal and intestinal epithelium was better than without LUM/IVA. Detailed medication response until 16 weeks after first intake of LUM/IVA can be seen in Table 1.

**Patient 4** (female, aged mid-20s, homozygous F508del-CFTR mutation) with mild CF lung disease and pancreas insufficiency at therapy start with LUM/IVA. Sweat chloride concentration, NPD and ICM scores indicated CF. In patient 4 concerning sweat glands, an improvement of sweat chloride level was observed but defined as mild. Patient 4 was improving under the combination therapy with LUM/IVA regarding CFTR function in intestinal epithelium. Faecal calprotectin remained low compared with day 0. Furthermore, BMI also improved during the study period, while CFTR function of the nasal epithelium was even worse than without LUM/IVA and no positive effect could be reached in lung function parameters. In contrast, an improvement of LCI$_{2.5\%}$ was seen and resulted in less inhomogeneity of the small airways. Detailed medication response until 16 weeks after the first intake of LUM/IVA can be seen in Table 1.

**Methods**

This prospective, single-centre monitoring pilot study was conducted to gain a detailed overview of CFTR function during the administration of different CFTR modulators in real-time settings.
Table 1. Data of several outcome parameters for patient 1 (#; coloured in orange), patient 2 (*) coloured in grey), patient 3 (~; coloured in yellow) and patient 4 (§; coloured in green) during the observational period of LUM/IVA intake (day 0 till week 16) are outlined below. The outcome parameters are divided in lung function data (spirometry, multiple breath washout), gastrointestinal aspects (BMI, faecal chymotrypsin and calprotectin) and CFTR bioassay data (sweat chloride and sodium, NPD scores, ICM outcome parameters).

| Day 0 | Week 2 | Week 4 | Week 16 |
|-------|--------|--------|---------|
| Patient 1 | Patient 2 | Patient 3 | Patient 4 | Patient 1 | Patient 2 | Patient 3 | Patient 4 |
| Day 0 | Week 2 | Week 4 | Week 16 |
| Patient 1 | Patient 2 | Patient 3 | Patient 4 | Patient 1 | Patient 2 | Patient 3 | Patient 4 |
| Day 0 | Week 2 | Week 4 | Week 16 |
| Patient 1 | Patient 2 | Patient 3 | Patient 4 | Patient 1 | Patient 2 | Patient 3 | Patient 4 |
| Day 0 | Week 2 | Week 4 | Week 16 |
| Patient 1 | Patient 2 | Patient 3 | Patient 4 | Patient 1 | Patient 2 | Patient 3 | Patient 4 |

- **BMI**, body mass index; **CFTR**, Cystic Fibrosis Transmembrane Conductance Regulator; **FRC**, functional residual capacity; **GLI**, global lung initiative; **ICM**, intestinal current measurements; **Isc**, short-circuit current; **LUM/IVA**, lumacaftor/ivacaftor; **NPD**, nasal potential difference; **ppFEV1**, percent predicted (pp) forced expiratory volume in 1 s; **ppFVC**, percent predicted (pp) results of forced vital capacity; **ppMEF25**, percent predicted (pp) mean expiratory flow at 25% of FVC.
Table 2. Data of several outcome parameters for patient 1 during the observational period of LUM/IVA (day 0 till week 16; #; coloured in orange) are outlined below. After treatment with LUM/IVA for 3 years, ELX/TEZ/IVA was started following the same prospective observational investigation (day 0 till week 16; °; coloured in blue). Results for day 0 of ELX/TEZ/IVA correspond to results three years after LUM/IVA. The outcome parameters are divided in lung function data (spirometry, multiple breath washout), gastrointestinal aspects (BMI) and CFTR bioassay data (sweat chloride and sodium, ICM outcome parameters). NPD measurements were not performed due to technical problems.

| Patient 1 | Day 0 | Week 2 | Week 4 | Week 16 |
|-----------|-------|--------|--------|---------|
|           | LUM/IVA # | ELX/TEZ/IVA ° | LUM/IVA # | ELX/TEZ/IVA ° | LUM/IVA # | ELX/TEZ/IVA ° | LUM/IVA # | ELX/TEZ/IVA ° |
| ppFEV₁ (GLI) | 46.1 | 53.7 | 41.3 | 62.2 | 45.5 | 59.3 | 53.4 | 62.8 |
| ppFVC (GLI) | 69.7 | 86.1 | 66.3 | 92.5 | 75.6 | 88.7 | 79.7 | 94.3 |
| ppMEF₂₅ (GLI) | 10.7 | 13.2 | 9.1 | 18.1 | 9.1 | 13.2 | 12.5 | 20.2 |
| FRC | 2.3 | 2.4 | 2.7 | 2.1 | 2.3 | 1.9 | 2.0 | 2.1 |
| LCI₂₅% | 22.9 | 15.7 | 21.1 | 14.8 | 21.6 | 13.7 | 23.0 | 13.8 |
| BMI [kg/m²] | 19.3 | 19.0 | 19.3 | 19.6 | 19.2 | 19.7 | 18.3 | 19.5 |
| Faecal chymotrypsin [U/g] | 13.2 | 14.2 | 16.0 | 22.7 | 36.5 | 10.8 | 68.8 | 22.4 |
| Faecal calprotectin [µg/g] | 430.3 | 163.5 | 86.6 | 0 | 83.4 | 18.3 | 402.3 | 63.5 |
| Sweat chloride right [mmol/L] | 100 | 61 | 55 | 14 | 54 | 14 | 47 | 11 |
| Sweat chloride left [mmol/L] | 100 | 59 | 42 | 11 | 52 | 16 | 45 | 8 |
| Sweat sodium right [mmol/L] | 109 | 40 | 67 | 29 | 61 | 30 | 51 | 24 |
| Sweat sodium left [mmol/L] | 111 | 47 | 50 | 23 | 62 | 35 | 60 | 17 |
| ΔIsc forsk/IBMX, carba + hist [µA/cm²] | 5.88 | 11.30 | 21.38 | 82.83 | 79.28 | 100.01 | 62.29 | 78.68 |
| best biopsy [µA/cm²] | 24.30 | 16.95 | 33.90 | 104.67 | 97.35 | 142.38 | 92.66 | 90.97 |

BMI, body mass index; CFTR, Cystic Fibrosis Transmembrane Conductance Regulator; ELX/TEZ/IVA, elexacaftor/tezacaftor/ivacaftor; FRC, functional residual capacity; GLI, global lung initiative; ICM, intestinal current measurements; Isc, short-circuit current; ΔIsc forsk/IBMX, carba + hist, sum of delta Isc forskolin/3-Isobutyl-1-methylxanthine (IBMX), delta Isc carbachol and delta Isc histamine; LCI, lung clearance index; LUM/IVA, lumacaftor/ivacaftor; NPD, nasal potential difference; ppFEV₁, percent predicted [pp] forced expiratory volume in 1s; ppFVC, percent predicted [pp] forced expiratory volume in 1s; ppMEF₂₅, percent predicted [pp] mean expiratory flow at 25% of FVC.
Table 3. Data of several outcome parameters for patient 2 during the observational period of LUM/IVA (day 0 till week 16; *; coloured in grey), during TEZ/IVA start (day 0 till week 16; +; coloured in yellow) and during ELX/TEZ/IVA intake (day 0 till week 16; ^; coloured in green) are outlined below. The outcome parameters are divided in lung function data (spirometry, multiple breath washout), gastrointestinal aspects (BMI) and CFTR bioassay data (sweat chloride and sodium, NPD scores, ICM outcome parameters). Faecal chymotrypsin and calprotectin was not analysed in patient 2.

| Patient 2 | Day 0 | Day 14 | Week 4 | Week 16 |
|-----------|-------|--------|--------|---------|
|           | LUM/IVA * | TEZ/IVA + | ELX/TEZ/IVA ^ | LUM/IVA * | TEZ/IVA + | ELX/TEZ/IVA ^ | LUM/IVA * | TEZ/IVA + | ELX/TEZ/IVA ^ |
| ppFEV1 [GLI] | 40.7 | 35.8 | 36.2 | 41.9 | 41.4 | 51.6 | 51.3 | 30.2 | 56.5 | 37.5 | 40.3 | 63.2 |
| ppFVC [GLI] | 62.9 | 59.9 | 64.7 | 66.8 | 72.3 | 79.2 | 78.9 | 50.9 | 88.3 | 65.4 | 67.0 | 91.4 |
| ppMEF25 [GLI] | 11.5 | 10.3 | 10.3 | 10 | 9.8 | 16.4 | 13.1 | 8.8 | 18.2 | 8.6 | 10.8 | 24.6 |
| FRC | 2.4 | 3.0 | 3.1 | 2.8 | 2.0 | 2.5 | 2.1 | 2.2 | 2.3 | 3.0 | 2.7 | 2.6 |
| LCI25% | 19.6 | 15.7 | 17.6 | 19.7 | 18.5 | 13.6 | 20.4 | 18.6 | 13.3 | 19.4 | 17.8 | 12.4 |
| BMI [kg/m²] | 18.3 | 18.7 | 19.5 | 18.2 | 18.8 | 19.9 | 17.9 | 18.7 | 19.4 | 18.3 | 19.4 | 19.0 |
| Sweat chloride right [mmol/L] | 105 | 113 | 112 | 99 | 113 | 52 | 95 | 114 | 58 | 122 | 113 | 58 |
| Sweat chloride left [mmol/L] | 104 | 109 | 120 | 98 | 112 | 50 | 95 | 114 | 58 | 108 | 111 | 55 |
| Sweat sodium right [mmol/L] | 117 | 130 | 123 | 113 | 121 | 60 | 113 | 125 | 62 | 133 | 120 | 63 |
| Sweat sodium left [mmol/L] | 119 | 129 | 132 | 118 | 123 | 59 | 120 | 123 | 66 | 123 | 126 | 61 |
| Mean recovery [mV] | -5.55 | -0.99 | -3.73 | -7.09 | -2.37 | -7.06 | 2.85 | 1.68 | -24.23 | 2.09 | 1.38 | -29.86 |
| [%] | [-13.03] | [-0.36] | [-19.18] | [-20.57] | [-9.29] | [41.95] | [11.61] | [5.23] | [-120.07] | [7.00] | [3.68] | [-125.31] |
| Wilschanski score | 0.88 | 1.0 | 0.83 | 0.81 | 0.91 | 0.66 | 1.12 | 1.05 | 0.3 | 1.07 | 1.04 | 0.29 |
| Sermet score | -1.79 | -1.24 | -0.56 | -0.96 | -1.02 | -0.06 | -1.54 | -1.79 | 1.66 | -1.72 | -2.03 | 2.09 |
| ΔI sc forsk/IBMX, carba + hist [µA/cm²] | 6.84 | -6.22 | 17.89 | 25.40 | 25.80 | 27.97 | 3.53 | 71.57 | 26.98 | 7.63 | 27.31 |
| best biopsy [µA/cm²] | 26.56 | 6.22 | 25.99 | 34.47 | 25.99 | 66.90 | 66.41 | 9.04 | 106.79 | 37.86 | 14.69 | 36.73 |

BMI, body mass index; CFTR, Cystic Fibrosis Transmembrane Conductance Regulator; ELX/TEZ/IVA, elексачафт/or tezачафт/or ivачафт/or; FRC, functional residual capacity; GLI, global lung initiative; ICM, intestinal current measurements; I sc, short-circuit current; ΔI sc forsk/IBMX, carba + hist, sum of delta I sc forskolin/3-Isobutyl-1-methylxanthine (IBMX), delta I sc carbachol and delta I sc histamine; LCI, lung clearance index; LUM/IVA, lumacaftor/ivacaftor; NPD, nasal potential difference; ppFEV1, percent predicted [pp] forced expiratory volume in 1 s; ppFVC, percent predicted [pp] results of forced vital capacity; ppMEF25, percent predicted [pp] mean expiratory flow at 25% of FVC; TEZ/IVA, tezачафт/or ivачафт/or.
For patients, monitoring was explained in detail and written informed consent was obtained before study start. The Ethical Committee for Human Research at the Medical University of Innsbruck approved this project.

Pulmonary function testing was performed with MasterScreen™ Body (Jaeger® or rather CareFusion®, Hoechberg, Germany) according to the spirometry standards of the American Thoracic Society/European Respiratory Society (ATS/ERS). Percent predicted (pp) results of forced vital capacity (ppFVC), forced expiratory volume in 1 s (ppFEV₁), mean expiratory flow at 25% of FVC (ppMEF₂₅), were based on equations of the global lung initiative (GLI by Quanjer et al.²¹).

Multiple Breath Washout (MBW) techniques were carried out using Exhalyzer® D (Eco Medics AG®, Duernten, Switzerland) with 100% oxygen, obtained functional residual capacity (FRC) and lung clearance index (LCl₂₅%).

Gastrointestinal parameters (faecal chymotrypsin and faecal calprotectin) were obtained from stool samples. The activity of chymotrypsin was used as parameter for the intake and resorption of pancreatic enzymes and was determined with Chymotrypsin Activity Kit® (Immundiagnostik AG®, Bensheim, Germany) and measured using Agilent Cary 60 UV-Vis® spectrophotometer (Agilent Technologies®, Santa Clara, California, USA) accordingly to manufacture’s manuals. Normal values are defined >6 U/g. Furthermore, calprotectin (a marker for intestinal inflammation)²²,²³ was determined with commercially available Enzyme-Linked Immunosorbent Assay (ELISA) Kit Calprest® (Eurospital diagnostic®, Trieste, Italy) and measured by VICTOR™ X3 Multilabel Reader (PerkinElmer®, Waltham, Massachusetts, USA) according to manual. Values <50 μg/g stool were considered normal.

Sweat tests were performed with pilocarpine iontophoresis. Two impregnated swabs with pilocarpine were placed on smooth and hairless region (i.e. forearm). Electrodes were added to receive continuous current flow (from cathode to anode). As a result, pilocarpine molecules migrated in the cutis and stimulated the sweat glands production. The amount of pilocarpine, current (1–4 mA) and duration of impact time are standardized. After 5 min swabs were removed. Sweat was collected for half an hour in a Macroduct® Sweat Collection System (Model 3700, Wescor®, Logan, Utah, USA). Afterwards, sweat was collected in an Eppendorf tube. For quality reasons, sweat test was performed on right and left arm. For measurement of chloride concentration, a Chloridimeter CM20 (Gonotec®, Berlin, Germany) and for sodium a Flame Photometer PFP7/C (Jenway®, Stone, UK) were used.

For NPD performance, we used a Calomel / Agar bridge. A double lumen catheter of Marquat Genie Biomedical® (Boissy-Saint-Léger, France) was placed on the nasal epithelium, resting against the surface of the target epithelium, and acting as an exploring electrode, whereas a 23G butterfly needle of Becton Dickinson® (Franklin Lakes, New Jersey, USA) filled with 3% agar and ringer solution was placed on the upper arm serving as reference electrode.²⁴ The bioelectric potential can be measured by using a high-impedance voltmeter [Power Lab® 8/35 with a BMA-200® AC/DC portable preamplifier and an ISO-Z® isolated head stage of AD Instruments® (Oxford, UK)] between these two electrodes [connected to REF 401 reference electrodes by Radiometer Analytical® (Villeurbanne, France)]. Measurements were performed according to the SOP® on both nostrils. Changes in potential difference (mV) by different solutions were recorded continuously by Lab Chart® [AD Instruments® (Oxford, UK)] and can be sorted out at defined points of interest. Closed loop offset initial and final as well as finger PD (pre, between nostrils and post) assess as quality criteria.

To evaluate the change in membrane potential and the function of the chloride channel, the recovery equation was used:

$$\text{recovery} = \Delta PD_0Cl - \text{Iso}(= \Delta 0Cl + \Delta Iso = \text{Isoproterenol} - \text{Amiloride})$$

in total and percent,

- **Wilschanski score**
  $$\text{Wilschanski score} = e \left( \frac{\sum \Delta 0Cl + \Delta Iso}{\Delta \text{Amiloride}} \right) = e \left( \frac{\text{Recovery}}{\Delta \text{Amiloride}} \right),$$

- **Sermet score**
  $$\text{Sermet score} = -0.11 \times \left( \sum \Delta 0Cl + \Delta Iso \right) - 0.05 \times \Delta \text{Amiloride},$$

where

- $\Delta PD_0Cl$ is the difference in potential difference before and after application of pilocarpine
- $\Delta 0Cl$ is the change in chloride concentration due to pilocarpine
- $\Delta Iso$ is the change in chloride concentration due to isoproterenol
- $\Delta Amiloride$ is the change in chloride concentration due to amiloride
- $e$ is the base of natural logarithms

[21] Quanjer et al.
[22] Wilschanski
[23] Sermet
were calculated. Values were rated as pathologi-
cal if recovery is > -8 mV in total and < -50%.
Furthermore, a Wilschanski score > 0.70 and
Sermet score < 0.27 were pathognomonic for
CFTR dysfunction.

The same current measurements as discussed
before can be performed on the intestinal epithe-
lum (ICM) ex vivo. Therefore, biopsy samples
of the rectum were extracted freshly by a suction
biopsy device [Trewavis Surgical® (Victoria,
Australia)] with a defined suction pressure of
9 psi/60 kPa for the least invasive procedure.
According to the SOP at least four biopsies
were collected. Rectal biopsies were obtained at
day 0 as well as 5 h after last dose of LUM/IVA at week
2, 4 and 16, respectively.

The biopsies are given in Meyler buffer solution
(128 mmol/L NaCl, 20.2 mmol/L NaHCO₃,
20.2 mmol/L Na₂HPO₄, 0.4 mmol/L NaH₂PO₄,
4.7 mmol/L KCl, 1.0 mmol/L MgCl₂, 1.3 mmol/L
CaCl₂, 10 mmol/L HEPES; pH 7.4, osmolarity
300 mOsm) shifted with p-glucose (0.18 g/100 ml)
and indomethacin [100 µl/100 ml; acts as cyclo-
oxigenase (COX) 1 and 2 inhibitor] to reduce basal
chloride secretion caused by endogenous produc-
tion of prostaglandins (cAMP, respectively) and
transported on dry ice. In the laboratory the biop-
sies were fixed into 1.5 or 1.2 mm diameter
(defined surface; 0.018 or 0.011 cm²) aperture
sliders, placed in Ussing chambers EM-CSYS-4
(sliders and chambers supplied by Physiologic
Instruments® (San Diego, USA), filled with pre-
warmed (37°C) Meyler buffer and were immedi-
ately connected to carbogen gas (95% O₂ and 5%
CO₂). The aperture was completely closed with
biopsy material and the apical and basolateral side
of the biopsy was identified before.

Subsequently, the voltage electrodes were placed
close to the tissue and the current electrodes were
attached at distance.

A system equilibration time of nearly 20–30 min
was considered. PD offset should be nearly 0 mV
and final fluid resistance compensation values
should range 250–350 Ω for the 1.5 mm diameter
aperture slider in open-circuit mode. After tissue
equilibration for around 5 min in open-circuit
mode, the system was switched to short-circuit
mode, basal PD (values around 0 mV) and basal
resistance (Rt; range of 15–30 Ω × cm²) of
tissue were recorded. Several different specific
stimulators of the chloride secretion were added
in an orderly manner to the apical (mucosal) and
basolateral (serosal) bathing solutions according
to the SOP. The short-circuit current (Isc) [µA/cm²] as a value
of ion transport was amplified with a Multi-
Channel Voltage Current Clamp and four pream-
plifiers [all supplied by Physiologic Instruments®
(San Diego, USA)] and recorded by Lab Chart®
[AD Instruments® (Oxford, UK)] through the
whole process of stimulation of CFTR-mediated
chloride secretion.

For evaluation, the sum of delta Isc forskolin/
3-Isobutyl-1-methylxanthine (IBMX), delta Isc
carbachol and delta Isc histamine (ΔIsc forsk/IBMX,
carba + hist) were calculated reflecting the function
of the chloride channel. In patients with CF
only a small amount of chloride was detected
(low ΔIsc forsk/IBMX, carba + hist), in non-CF a huge
current comparatively was detected (high ΔIsc
forsk/IBMX, carba + hist). Minso et al.20 set the ΔIsc forsk/
IBMX, carba + hist cut-off level with 39 µA/cm² for
detecting CF.

Measurements of NPD and ICM are undertaken
throughout only a few CF centres in Europe. We
implement NPD and ICM for 5 years now and
established a CFTR function laboratory the
results of which we discuss regularly with another
CF reference centre.

For gaining high-quality data, NPD and ICM
data of healthy individuals (n = 4) as well as results
of CF patients without CFTR modulator (day 0;
n = 4) were compared with literature and are
presented in the supplementary material.

Discussion
Defining a prognosis for patients with CF is
exceedingly difficult due to the huge geno–phe-
notype variability. Even with the same mutation
e.g. F508del), the clinical outcome can range
from severe progression and involvement of sev-
eral organs to mild courses. With conservative
symptomatic therapy including inhalation, sports,
nutrition and medication the progression of ill-
ness can be influenced effectively. New developed
therapies started with the goal to find at least a
causal determined therapy approach with muta-
tion specific therapies in the last years. This goal
was reached partially by the admission of CFTR
modulator therapies. F508del leads to a reduction in CFTR processing and transport to the cell surface. Therefore, addressing the underlying cause of disease in patients homozygous for this mutation is complex. This is expected even more from CFTR modulator therapies. Our observation shows that even with the same mutation (F508del-CFTR), sex, age and disease severity, the clinical outcome after starting the combination therapy with LUM/IVA ranges from nearly complete treatment response (patients 1 and 3) and organ-specific rescue of CFTR function (patient 4) to non-responding therapy outcome (patient 2).

The results of the pivotal registration studies for LUM/IVA showed a mean relative difference of FEV1 between active treatment and placebo of 4.3–6.7% at week 24, whereas another study by Graeber et al. only found a change of 2.27% after 8–16 weeks. In fact, the difference between receiving LUM/IVA or placebo regarding FEV1 was little, smaller than expected and comparable to other therapy approaches like long-term exercise for 6 months independent of strength or endurance training.

We found a difference in FEV1 comparing day 0 and week 16 of 7% in our observation of LUM/IVA therapy. Focusing on TEZ/IVA in patient 2, a benefit in lung function parameters was seen during the observational period, but it was not constant (Table 3). Whereas our observation of ELX/TEZ/IVA response showed a huge improvement in lung function parameters even 2 weeks after initiation and further improvement could be achieved 16 weeks after start in patients 1 and 2 (Tables 2 and 3). So, it might be enough to focus on lung function parameters as primary endpoint approaches like long-term exercise for 6 months independent of strength or endurance training.

A more sensitive parameter for early decline of lung function and an indicator for ventilation inhomogeneity or air trapping is the lung clearance index (LCI2.5%). An Irish working group showed even lower median LCI2.5% levels post-LUM/IVA treatment and demonstrated the positive effect of a LUM/IVA therapy in adolescence. These results were consistent with our findings for LUM/IVA, although our patient cohort was older than the Irish one, because nearly every patient (despite patient 1) had a lower LCI2.5% at the end of the observation. Focusing on the results of all analysed parameters of patient 1 (improving in all 3 CFTR function bioassays sweat test, NPD and ICM as well as FEV1 during the study period), LCI2.5% is not the most sensitive outcome parameter for LUM/IVA therapy evaluation as it is suggested by this working group, although it might be helpful in patients with a FEV1 ≥80% for detecting early lung disease progression. In patient 2, no improvement of LCI2.5% could be achieved by TEZ/IVA, but post-ELX/TEZ/IVA treatment an obvious decline in LCI2.5% was seen. However, if a CFTR modulator therapy is started early in life and LCI2.5% is low, structural pulmonary damage could be avoided or development might be reduced and delayed than without modulator therapy.

Focusing on other clinical endpoints like BMI, previous studies for LUM/IVA found an improvement in BMI in their patients and an increase in BMI is a possible and sometimes desired side effect. In contrast to literature, BMI showed no constant improvement in our patients when evaluated pre- and post-LUM/IVA. A reason for that might be that our patients had an appointment with dieticians at study start to avoid an excessive increase in body weight. Therapeutic interventions were set where BMI was already over the target range.

Furthermore, faecal calprotectin – a marker of inflammation in the intestinal tract – showed an improvement in intestinal inflammation focusing on Ivacaftor and more recently LUM/IVA lead to lower faecal calprotectin levels in French adolescents. Elevated concentrations are associated with disease severity of CFTR mutation, pancreas insufficiency and progression. In fact, F508del homozygous subjects have higher faecal calprotectin than patients with other mutations. In our cohort, only patient 1 showed increased faecal calprotectin at day 0. Nevertheless, no obvious modification of faecal calprotectin levels after LUM/IVA initiation were found in our small cohort, even though our cohort was older than the French patients and elevated concentrations are associated with disease progression.

In further studies, it is necessary to evaluate the therapy response by focussing on pathophysiological processes due to CFTR functioning tests.
especially for LUM/IVA and TEZ/IVA treatment. We found a huge drop in sweat chloride concentration when comparing the results before LUM/IVA start and at week 2 or at week 4 (Table 1). Focussing on long-term effects, there is a reduction of 35.3 mmol/L analogue at week 16 of LUM/IVA treatment. Comparing our results with those of Graeber et al., we even achieved higher CFTR rescue focussing on sweat gland function. An obvious therapy effect concerning sweat chloride concentration was seen when ELX/TEZ/IVA was started in patients 1 and 2 (reduction of 50.5 mmol/L; Table 2 and 59.5 mmol/L; Table 3) consistent with reductions of sweat chloride concentration of 61.0 mmol/L according to literature.41 Guimbellot et al.42 argued that the sweat gland epithelium is not affected by sequelae of the defect such as inflammation or tissue destruction and therefore represents an objective parameter to measure CFTR dysfunction. However, CFTR function in sweat gland is not correlating with CFTR rescue in nasal or intestinal epithelium.30 In contrast, the NPD measurements are influenced by inflammation of the mucosa, nasal polyps and cooperation of the patient. Comparing NPD parameters (e.g. basal PD) our results match with data in the literature. Although histological fundamentals may conclude that the epithelium of the Regio respiratoria is correlating with the bronchus epithelium, it is not predictable if there is a correlation of an improvement in NPD with lung function parameters.45 However, our results of patient 1 show that there were lower NPD scores after the intake of LUM/IVA, but the lung function parameters did not improve before week 16 and even first declined. Moreover, CFTR rescue in the nasal epithelium is specified by Graeber et al.30 with 10.2% after 8–16 weeks, whereas we only found 4.4% at study end point with LUM/IVA treatment, even though there was a huge improvement in CFTR function at first (Table 1). Graeber et al.30 did not measure CFTR function at defined study points. Consequently, the comparability of their results lacks due to missing data. The long-term effect on the nasal epithelium by the CFTR modulator therapy with LUM/IVA in our pilot study was moderate. These findings are confirmed by the modifications of lung function parameters. However, results of patient 2 suggest that post-ELX/TEZ/IVA treatment NPD scores were in a normal range (Table 3). These results regarding NPD parameters post-ELX/TEZ/IVA were confirmed by the study of Graeber et al.41 focusing on more than 100 patients.

Furthermore, the effect on CFTR function by modulator therapy could be evaluated by ICM and showed an improvement of 17.7% evaluating the response of \( \Delta I_{sc \text{ forsk/IBMX, carba + hist}} \) before and after initiation of LUM/IVA therapy.30 Although our study sample was small, significant changes of \( \Delta I_{sc \text{ forsk/IBMX, carba + hist}} \) in the best responding biopsy were found. An obvious changing of CFTR function in the intestine (best responding biopsy) post-LUM/IVA treatment was found at week 4 (fivelfold higher \( I_{sc \text{ forsk/IBMX, carba + hist}; Table 1} \) as well as post-ELX/TEZ/IVA intake (Tables 2 and 3). In contrast to literature, patient 2 showed no improvement post-TEZ/IVA treatment and highest \( \Delta I_{sc \text{ forsk/IBMX, carba + hist}} \) was seen at week 2 (Table 3). ICM is one of the most sensitive outcome parameters to evaluate CFTR function and restoration of CFTR function due to modulator therapy.

In fact, conventional outcome parameters (like sweat chloride concentration, FEV\(_1\), LCL\(_{2.5%}\), and BMI) are usually reliable and valid for the evaluation of treatment response, but in the case of non-responders an improvement of clinical parameters is lacking. No correlations of ppFEV\(_1\) or BMI with CFTR bioassays were found recently, indicating inadequate outcome parameters for detecting response to modulators at the level of the underlying defect. Nevertheless, CFTR function tests are performed in very few centres and cannot be offered to every patient, which causes a huge limitation. Nonetheless, functional CFTR biomarkers focusing on different organs (e.g. NPD, ICM) can help clinicians potentially to distinguish responders from non-responders especially post LUM/IVA or -TEZ/IVA treatment. Therefore, it should be considered in selected cases for gaining additional information on pathophysiological conditions. For example, if there is no response to LUM/IVA or TEZ/IVA treatment CFTR bioassays should be performed before switching to ELX/TEZ/IVA. Furthermore, we recommend repeating CFTR bioassays after the start of ELX/TEZ/IVA intake, if an improvement of FEV\(_1\) lacks.

So, these diagnostic tests are not only recommended for confirming or excluding a CF diagnosis,11 but they might also be an important tool in clinical trials and for the prediction of patient’s outcome.46 Due to lacking data on long-term effects, Rubin et al.47 recently published their modelling study and their analysis showed that
the combination therapy of LUM/IVA increased the survival with higher lung function and lower risk of lung transplantation.

However, to account for the long-term consequences, we focused on improvement of clinical outcome parameters and negative side effects. Due to the small number of patients included in our monitoring study no significant alterations either in CFTR biomarkers nor in conventional clinical outcome parameters could be found after LUM/IVA. Furthermore, results emphasize that non-responders for LUM/IVA and TEZ/IVA exist, and such conclusions could not be drawn, if there would not be a strict and regular monitoring emphasizing on several outcome parameters.

So, in case of non-responders (like patient 2), LUM/IVA and TEZ/IVA treatment should be stopped due to possible side effects and cost-effectiveness ratio. Consequently, treatment might be switched to other available highly efficient medication therapies.

Conclusion

This is the first personalized monitoring study of different CFTR modulator efficacy at standardized appointments (day 0, week 2, 4 and 16), which has focused on several routine clinical outcome parameters as well as CFTR bioassays. We showed that there are differences of CFTR function before and after starting the intake of CFTR modulators as well as organ-specific changes due to the therapy. Even with the same mutation (F508del-CFTR), sex, age and disease severity, the clinical outcome ranges from nearly complete treatment response (patients 1 and 3) and organ-specific rescue of CFTR function (patient 4) to non-responding therapy outcome (patient 2). In fact, conventional used outcome parameters (like FEV₁ and BMI) are usually reliable and valid for the evaluation of CFTR modulator response, but in the case of non-responders (especially post-LUM/IVA or -TEZ/IVA treatment) clear improvement of clinical parameters is lacking and data on CFTR function of different organs (NPD, ICM) can confirm or disprove ongoing CFTR dysfunction. Non-responders for ELX/TEZ/IVA were not found in our cohort. Therefore, functional CFTR biomarkers should be considered in selected cases for gaining additional information on pathophysiological conditions.

Nevertheless, real-life modulator treatment protocols may need to be adapted individually according to observational results (e.g. therapy discontinuation, switching of CFTR modulators).

Therefore, several clinical and functional biomarkers on multiple defined occasions could be helpful to evaluate individual treatment response in each patient treated with modulators.

Declarations

Ethics approval and consent to participate

This observational pilot study was approved by the Ethics Committee of the Medical University of Innsbruck (Austria) by 9 February 2016: classification number AN2015-0227 353/2.5; EudraCT-Nr. 2015-00380741. Informed consent was obtained by participants.

Consent for publication

Written informed consent for publication of clinical details was obtained from patients.

Author contributions

Katharina Niedermayr: Conceptualization; Data curation; Investigation; Methodology; Writing – original draft.
Verena Gasser: Investigation; Methodology; Writing – review & editing.
Claudia Rueckes-Nilges: Investigation; Methodology; Writing – review & editing.
Dorothea Appelt: Investigation; Methodology; Writing – review & editing.
Johannes Eder: Conceptualization; Methodology; Writing – review & editing.
Teresa Fuchs: Conceptualization; Methodology; Writing – review & editing.
Lutz Naehrlich: Investigation; Methodology; Writing – review & editing.
Helmut Ellemunter: Conceptualization; Funding acquisition; Methodology; Writing – review & editing.

Acknowledgements

The authors thank the patients with cystic fibrosis for their participation in this observational pilot study. Furthermore, we thank Mrs Nikelwa Theileis, MA, for proofreading.

Funding

The authors received no financial support for the research, authorship and/or publication of this article.
Competing interests
The authors declared the following potential conflicts of interest with respect to the research, authorship and/or publication of this article: K.N. reports support for attending meetings and/or travel by Chiesi Pharmaceuticals GmbH and TEVA-Ratiopharm Arzneimittel Vertriebs-GmbH, outside the submitted work. V.G. and C.R.-N. have nothing to disclose. D.A. reports support for attending meetings and/or travel by Mylan Austria GmbH, Vertex Pharmaceuticals and TEVA-Ratiopharm Arzneimittel Vertriebs-GmbH, outside the submitted work. J.E. reports support for attending meetings and/or travel by Corbus Pharmaceuticals GmbH and Chiesi Pharmaceuticals GmbH. T.F. reports support for attending meetings and/or travel by Mylan Austria GmbH and Vertex Pharmaceuticals, outside the submitted work. L.N. reports grants or contracts from German Center for Lung Research, Vertex Pharmaceuticals and Boehringer Ingelheim (for study participation), outside the submitted work. Furthermore, he is a member of the trial steering committee for CF STORM, the medical leader of the German CF registry as well as the manager of the pharmacovigilance study of the ECFS and editorial supporter of Articulate Science LLC. H.E. reports grants or contracts with Vertex Pharmaceuticals (for study participation), outside the submitted work. Furthermore, he received personal support for presentations and for advisory board by Vertex Pharmaceuticals as well as by TEVA-Ratiopharm Arzneimittel Vertriebs-GmbH and Chiesi Pharmaceuticals support for attending meetings.

Availability of data and materials
The datasets during and/or analysed during the current study available from the corresponding author on reasonable request.

ORCID iD
Katharina Niedermayr https://orcid.org/0000-0001-7195-3632

Supplemental material
Supplemental material for this article is available online.

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