to CFTR-modulators. Several laboratories across the world are adopting the organoid model. Quality and repeatability of the assay are important to ensure the validity of results across laboratories. Therefore, procedures have to be standardized to aim for minimal variability in outcome between labs. The aim of this study is to standardize procedures and to detect, describe and tackle the causes of variability in the assay.

Methods: To standardize working procedures, we first exchanged protocols from the currently existing organoid laboratories (UMC Utrecht, KUZ Leuven and University of Lisboa). Next, we detected differences in protocols and mutually decided which working procedure was preferred. We merged the protocols into one European standard protocol. We exchanged organoids with genotypes ranging from severe to wild-type across the three laboratories. We will perform the Forskolin-induced-swelling (FIS) assay with several different measuring conditions (locally produced growth medium, identical growth medium, and after identical and different amounts of passages. Results of the FIS assay will be compared to detect differences in handling, growth medium and other factors, and their effect on assay variability. Subsequently, important factors that influence variability will be adapted in the protocol.

Results: A European standard protocol has been generated. This protocol has been implemented across three laboratories. Measurements are currently taking place. The results of these analyses will be presented at the conference.

Conclusion: The organoid model will be validated in three different laboratories, and one final protocol will be established to ensure quality and standardization of measurement outcomes.

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Creation of a tissue biobank to investigate CFTR biology and develop a personalized medicine approach to CF

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Recent advances in treatment for patients with cystic fibrosis (CF) have highlighted the need to collect tissues for personalized medicine. Currently, human bronchial epithelial cells (HBE) which are obtained during lung transplants, are extensively used in basic and translational research. The high variability observed in baseline function and response to treatments in cultured HBE have been attributed to inter-donor variability, however the factor(s) responsible for the variability have yet to be defined. Indeed, GWAS studies indicate several possible loci connected to CF and these genetic modifiers may be partially responsible for the modulation of CF pathology. In addition, the amount of cells collected is finite, which can limit the scope of the investigational studies. Nasal epithelial cells (NEC) have already proven to be an alternative source of cells expressing CFTR and have been used as a tool to better understand CFTR biology. When the NEC's are grown under air-liquid interface conditions, the function of CFTR can also be studied, similarly to the HBE's. Furthermore, NEC's can be collected by non-invasive techniques which make them an ideal renewable source of cells to establish a biobank. It is for all of these reasons which make NEC's very attractive for drug discovery. At Proteostasis, we are establishing a biobank of NEC's from healthy volunteers (HV) and patients with CF. We have developed a process which includes the following steps: (1) identify HV and CF patients, (2) collect NEC specimens, (3) ship samples and assess cell viability, (4) expand NEC to bank multiple vials and (5) thaw vials for testing. Our pilot studies have demonstrated the importance of proper collection techniques and shipping conditions to obtain viable NEC's. We are now in a situation to assess inter and intra-patient variability of cells obtained from both HV and CF patients.

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Distinguishing properties of CFTR potentiator FDL176

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FDL176 is a novel CFTR potentiator that stimulates chloride current in F508del-CFTR primary CF airway epithelial cells with similar *in vitro* efficacy to ivacaftor and an EC_{50} of 127 nM. In FRT cells expressing F508del-CFTR, stimulation with FDL176 increases chloride current >2-fold suggesting the open probability of uncorrected F508del-CFTR increases at least 2-fold upon acute exposure. The activity of FDL176 and ivacaftor following acute treatment was compared in other CF causing mutations expressed in FRT cells. In G551D cells, FDL176 increases chloride current by 10-fold and ivacaftor by 13-fold. In N1303K cells, potentiation with FDL176 and ivacaftor increase chloride current by 3-fold and 4-fold, respectively. Both potentiators increase chloride current by 2-fold in R117H cells and 1.2 to 1.3-fold was observed for mutations G85E, E92K, R560T. The potency of both potentiators was similar across different mutations, except G551D and N1303K where the potency was 10-fold higher. In lumacaftor corrected F508del-CFTR primary cells, chronic exposure to ivacaftor (≥6 hours) reduced chloride current by >50% compared to acute conditions (0 hrs); chronic exposure to FDL176 reduced chloride current by ~25%. The reduction of chloride current does not change in presence of human serum, but the IC_{50} of both potentiators shifts to higher concentrations. HRP cell surface expression in CFBE cells is not affected by acute exposure to FDL176 or ivacaftor, but prolonged exposure reduces amount of CFTR at the cell surface by 36% for FDL1176 and 57% for ivacaftor. In summary, FDL176 and ivacaftor produce a similar *in vitro* response upon acute stimulation, while the impact of chronic exposure to FDL176 on chloride current is less than ivacaftor.

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CFTR NBD2 mutant N1303K associates with and influences the functioning of the autophagosome

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Objectives: Severe disease-causing mutations also occur in NBD2.

Methods: To provide information on potential therapeutic strategies for mutations in NBD2, we studied the disease-causing NBD2 mutant, N1303K.

Results: Adding C4 + C18 produces a large increase in the B band of N1303K indicating that they stabilize the B band. To evaluate the effect of C4 + C18 on function, we measured Isc and found that C4 + C18 increases the currents approximately 4 fold. Next, we applied E64 and observed that N1303K was not sensitive to E64. But further investigation showed that when C4 + C18 is applied, N1303K became sensitive to E64. To assess whether autophagy is involved with N1303K degradation, we evaluated the ratio LC3-II/I and the absolute amount of LC3-II. There is no statistically significant increase in either the ratio of LC3-II/I or the absolute amount of LC3-II when E64 is applied in the absence of the corrector combination. This surprising result indicates that the rate of autophagy is slow in the cells containing N1303K. A large increase in the ratio of LC3-II /I and the absolute amount of LC3-II in the presence of C4 + C18 indicated that the correctors are increasing autophagy. Next, we performed confocal experiments to evaluate the degree of colocalization between the NBD mutants and the LC3 as a marker for autophagosome. Because LC3-II is associated with the autophagosome and LC3-1 is cytosolic we argued that colocalization between LC3 and N1303K, a membrane protein would represent the degree to which both proteins reside in the autophagosome membrane. We found significant overlap in location between LC3 and N1303K in untreated cells. The colocalization is reduced by the combination of correctors.

Conclusion: We conclude that correctors have a dual effect particularly on N1303K. They improve trafficking and function at the plasma membrane, but at the same time increase degradation via the autophagosome and lysosome system.

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CFTR modulators alter innate immune responses by primary airway epithelial cells challenged with rhinovirus

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Objectives: Airway epithelial cells (AEC) from CF lungs respond to viral infection with poor innate immune responses. The contribution of defective CF transmembrane conductance regulator (CFTR) channel function in these responses is not known. Here, we investigated whether CFTR correcting drugs could improve responses by CF AEC to human rhinovirus (HRV).

Methods: Primary AEC were obtained from 11 children with CF (five Class II, six Class III mutations) and six healthy, non-CF controls by airway brushing. Primary cultures were expanded by conditional reprogramming before infection with HRV1b (multiplicity of infection: 12.5) for 24 hrs. To compare the effect of CFTR modulation on anti-viral responses, AEC pre-treated for 72 hrs with lumacaftor and/or ivacaftor and then infected with virus were compared with AEC infected with virus alone. Supernatant was assessed for interferons (IFNβ, $IFN\lambda_1$ and $IFN\lambda_2$) and inflammatory cytokines (IL-6, IL-8, RANTES) by ELISA.

Results: In non-CF AEC, HRV1b infection resulted in significantly increased IL-8 and RANTES ($p<0.05$), but not IL-6, IFNβ, $IFN\lambda_1$ or $IFN\lambda_2$. In addition, responses did not significantly change in the presence of CFTR modulators. In contrast, AEC of both CF genotypes generated IL-8 and IL-6 responses to HRV1b ($p<0.05$). With CFTR modulator treatment however, IL-6 production post infection was not significantly elevated. Combined CFTR therapy for Class II CF was also found to reduce RANTES production ($p<0.05$). IFN responses by CF AEC to HRV1b infection were mixed, with minimal levels detected following infection except for $IFN\lambda_1$, which was significantly elevated ($p<0.05$). However, this was no longer significant after CFTR modulator therapy for both Class II and Class III ($p<0.05$).

Conclusion: CFTR therapies were found to reduce some inflammatory responses by CF AEC to HRV1b, but also modulate $IFN\lambda_1$. We are currently analysing RNAseq data to compare innate immune gene networks between treatments and phenotypes.

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Analysis of the airway surface liquid pH in pediatric patients. Pilot study

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Objectives: CFTR is a complex ABC protein that acts as a chloride channel and regulates the function of other membrane channels. The mutation of the gene produces a missing or dysfunctioning CFTR protein, which leads to an electrolytic disbalance in the apical membrane of the epithelial cells, giving an ionic alteration: hyposecretion of chloride and bicarbonate with sodium hyperabsorption.

There is experimental evidence that CFTR, in addition to being a cAMP-dependent chloride channel, may have other functions, for example the transport of bicarbonate and the regulation of other endogenous channels of chloride and calcium.

Some studies have proposed that if the acidic pH of the airway liquid surface (ASL) decreases its bactericidal capacity and increases the viscosity of mucus, increasing this pH, bacterial infections of the airway could be diminished.

The aim of this study was to analyze the pH of the ASL of 6 pediatric patients with a cystic fibrosis (CF) diagnosis and 2 non-CF pediatric patients.

Methods: Prospective, cross-sectional, descriptive study developed in 8 pediatric patients attended at the Pediatric Allergy and Pulmonology Unit of the Sabadell's hospital.

Induced sputum samples were collected in 8 patients (6 CF and 2 non-CF) between 6 and 16 years. The pH of the ASL was analyzed by pHmetry. Patients were randomly selected according to the clinical therapeutic needs of performing the induced sputum in each of them.

Results: From the CF population, a sample of 6 patients was obtained; the mean pH in the ASL was 6,746. From the non-CF, a sample of 2 patients (Dx ciliary dyskinesia) was obtained, whose average pH in ASL was 6.83.

Conclusion: In CF pediatric patients, the pH of the ASL could be acidic due to the defect in CFTR activity.

We recommend extending the study to a more significant sample.

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Nasal potential difference in βENaC-overexpressing mouse reveals pH-sensitive channel hyperactivity and shift of subunits stoichiometry

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Objectives: To characterize transepithelial nasal potential difference in β-ENaC overexpressing mouse model engineered to mimic CF airway disease.

Methods: We used transepithelial potential difference (PD) to measure ion transport across the nasal mucosa of wild-type and *scnn1b-Tg* heterozygous (Tg/+) mice. The following parameters were recorded: (1) the most negative basal PD value; (2) change in PD after nasal perfusion with buffered Ringer's solution at pH 7.4 but no change with pH 6.5; (3) decrease in PD after perfusion with Ringer's solution containing 10^{-4} M amiloride; (4) subsequent changes in PD after perfusion with Ringer's solution in which Cl^- was replaced by gluconate, and amiloride and 10^{-5} M forskolin were added. To test the hypothesis that elevated Na^+ transport in Tg/+ mouse airways is due to an increased number of αβγENaC channels or a possible change in subunits stoichiometry, we analyzed ENaC subunit transcripts in mouse nasal epithelium and in nasal epithelial cells in 3D primary cultures.

Results: More polarized basal PD values were recorded in Tg/+ than in wild-type mice. In Tg/+, but not in wild-type, perfusion of Ringer's-pH 7.4 rapidly induced a small depolarization (+4mV) that was prevented by adjusting the pH to 6.5. The response to amiloride was significantly increased (+50%) in Tg/+ compared to wild-type mice. Perfusion of chloride-free containing forskolin induced a hyperpolarization of similar magnitude in Tg/+ and in wild-type mice. Both α and βENaC transcript levels were elevated in nasal cells derived from Tg/+ mice with no significant change in γ-subunit expression.

Conclusion: Our data indicate that the CFTR-dependent chloride conductance is not affected by ENaC hyperactivity in mice. We showed here a pH-sensitive hyperactivity of ENaC in nasal epithelium of *scnn1b-Tg* mice possibly associated with a switch in subunit stoichiometry of the ENaC channel. However, the possible role of inflammation should also be considered.

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Mitochondrial dysfunction in cystic fibrosis

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Background: Cystic fibrosis (CF) is a chronic, progressive disease, caused by a mutation of the gene encoding for the CF Transmembrane Conductance Regulator (CFTR). Animal and cell studies have identified consequences of CFTR dysfunction, including reduced mitochondrial complex activity independent of chloride channel function. It is likely that mitochondrial complex I and complex IV are defective in cystic fibrosis patients, however this has not been proven in vivo. CFTR dysfunction has been linked to an increase in reactive oxygen species which alters the cellular redox status, may trigger apoptotic events and produce inflammatory responses that may affect innate immunity.

Objectives: To compare oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) in CF patients and control subjects. To use OCR is an indicator of mitochondrial respiration and extracellular acidification rate (ECAR) as an indicator of glycolysis.

Methods: Seven control and 6 homozygous F508del CF patients were recruited to donate one blood sample. Using density gradient separation, neutrophils were removed from residual PBMCs, oxygen consumption and glycolysis were measured using the Seahorse XF analyser by the addition of various inhibitors of the oxidative phosphorylation pathway in the PBMCs of each cell population. This provided measures of cellular activity including basal cell respiration, maximal oxygen consumption, non-mitochondrial cellular respiration and extracellular acidification rate.

Results: Results indicate control subjects have higher stimulated maximal oxygen consumption compared with CF subjects by 24% ($p<0.1$). Basal and