

Program and Abstracts



17th Scientific Meeting

**Organoids as models for disease and
treatment in CF**

September 21st + 22nd 2017
Schloss Waldhausen/Mainz

Organization

Scientific advisory board of the German Research Community for Cystic Fibrosis (FGM)
& Mukoviszidose Institute

Chairs

Nico Derichs (Berlin/Germany)

Silke van Koningsbruggen-Rietschel (Köln/Germany)

Burkhard Tümmler (Hannover/Germany)

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Program

Thursday, September 21st

- 11:30 am Get together + Lunch
- 12:30 pm Opening of the meeting/Introduction
Stephan Kruij (Chairman of the German CF association)
Manfred Ballmann (Chairman of the supervisory board Mukoviszidose Institut)
- 12:45 pm Sessions I: Sets the line, defines state of the art
Moderation: Nico Derichs / Manfred Ballmann
Hugo de Jonge (Rotterdam/Netherlands)
Chloride and bicarbonate transport in intestinal organoids: Differential effects of CFTR modulators and mutations
Meike Hohwieler (Ulm/Germany)
Human pluripotent stem cell-derived pancreatic organoids to study cystic fibrosis in a dish
Katherine Benson McCauley (Boston/USA)
Generation of human pluripotent stem cell-derived airway organoids to model cystic fibrosis in vitro
- 2:45 pm Coffee break + free discussion
- 3:15 pm Abstract-Session I: Organoids and CF research funding
Moderation: Nico Derichs / Manfred Ballmann
Marcel J.C. Bijvelds (Rotterdam/Netherlands)
CFTR-dependent anion and fluid transport in human bile duct-derived organoid cultures
Giovana Bavia Bampi (Hamburg/Germany)
The effect of silent single-nucleotide polymorphism (sSNP) on CFTR expression
Aarne Fleischer (Esporles/Spain)
Intestinal organoids from genome-edited CFTR iPS cells
Paola Melotti for Anna Baruzzi (Verona/Italy)
Intestinal epithelial organoids contribute to supporting drug development and diagnosis
Vincent Gulmans (Baarn/Netherlands)
HIT CF: a successful research program on personalised medicine through collaboration with patients, clinicians and researchers
- 4:30 pm Coffee break + free discussion
- 5:00 pm Keynote + Discussion
Moderation: Burkhard Tümmler / Silke van Koningsbruggen-Rietschel / Nico Derichs
Jeffrey Beekman (Utrecht/Netherlands)
Intestinal organoids, fluid secretion and cystic fibrosis: the past, present and future
- 8:00 pm Dinner

Friday, September 22nd

- 8:00 am Sessions II: Impact for CF
Moderation: Silke van Koningsbruggen-Rietschel / Helge Hebestreit
Kris De Boeck (Leuven/Belgium)
Use of organoids in clinical studies
Katherine Tuggle (Bethesda/USA)
CFF perspective on ex vivo models as tools for predicting CFTR modulator response
Panel Discussion
- 9:45 am Coffee break + free discussion
- 10:00 am Abstract Sessions II: Basic CF defect and pharmacological correction
Moderation: Silke van Koningsbruggen-Rietschel / Helge Hebestreit
Manfred Frey (Mannheim/Germany)
Bicarbonate and chloride transport assays using fluorescent biosensor cell lines for identification of novel synthetic anion transporters
Claudio Sorio (Verona/Italy)
Setup of a simplified method to measure CFTR-dependent iodine transport: HS-YFP assay
Paola Melotti (Verona/Italy)
Testing CFTR function in vivo by imaged ratiometric measurement of beta adrenergic/cholinergic sweat rate in human sweat glands
- 10:45 am Coffee break + free discussion
- 11:00 am Abstract Sessions III: Basic CF defect and infectiology
Moderation: Burkard Tümmler/Anna-Maria Dittrich
Adriely Góes (Saarbrücken/Germany)
Clarithromycin-loaded nanoparticles to treat M. abscessus lung infection
Cristiane Carvalho-Wodarz (Saarbrücken/Germany)
Modelling cystic fibrosis infected airways with a 3D in vitro model of human bronchial epithelia and macrophages to evaluate anti-infective formulations
Iwona Pranke (Paris/France)
Evaluation of the primary human nasal epithelial cell cultures as a biomarker in personalized Cystic Fibrosis treatment
- 11:45 am Coffee break + free discussion
- 12:00 am Session III: Perspectives
Moderation: Burkard Tümmler/Anna-Maria Dittrich
Ulrich Martin (Hannover/Germany)
Induced pluripotent stem cells for identification of novel drug combinations targeting cystic fibrosis lung and liver disease
David Sheppard (Bristol/UK)
Multiple impacts of rare mutations on CFTR function
- 1:20 pm Closing of the meeting
- 1:30 pm Lunch

Sessions I: Sets the line, defines state of the art

Chloride and bicarbonate transport in intestinal organoids: differential effects of CFTR modulators and mutations

Marcel Bijvelds, Kelly Meijsen, Maikel Peppelenbosch, Jeffrey Beekman, Inez Bronsveld, Anna Baruzzi, Sara Calderer, Claudio Sorio, Paola Melotti, Luca Frulloni, [Hugo de Jonge](#)

Most physiological assays currently used for CF diagnosis and as a biomarker in clinical trials of CFTR correctors and potentiators (NPD; ICM; FIS) measure CFTR-dependent anion transport but fail to discriminate between the chloride (Cl) and bicarbonate (HCO₃) component. Recent studies in CF animal models reveal that defective HCO₃ rather than Cl transport is the primary cause of impaired mucociliary clearance in the airways and luminal obstruction in the GI tract, major hallmarks of CF. However the role of HCO₃ in human CF pathogenesis, the impact of specific CFTR mutations on HCO₃ transport, and the ability of CFTR modulators to improve HCO₃ secretion in human CF epithelia have been poorly investigated and need further study.

Here we choose to study features of HCO₃ transport, including its restoration by clinically applied CFTR modulators, in a readily accessible tissue, i.e. rectal biopsies and 3D and 2D cultures of rectal organoids obtained from healthy controls (HC) and CF patients. We also aimed to validate the concept that CFTR functional variants associated with pancreatitis and sinusitis but not CF have a specific HCO₃ permeation defect.

To reach these goals, Cl and HCO₃ transport in HCs, CF patients (e.g. F508del; S1251N) and non-CF pancreatitis patients (D1152H) were compared at the level of rectal biopsies and 3D and 2D intestinal organoids using a variety of techniques, including short circuit current measurements in high Cl/low HCO₃ vs. low Cl/high HCO₃ media in Ussing chambers, pH-Stat measurements and forskolin-induced organoid swelling assays (FIS). Moreover the ability of WNK/SPAK kinases to switch CFTR from a Cl into a HCO₃ conductive state was assessed by WB of phospho-SPAK and the use of SPAK inhibitors.

The results of this ongoing study so far reveal that (1) HCO₃ secretion, but not Cl transport, is severely impaired in rectal biopsies of D1152H/F508del patients; (2) the WNK-SPAK pathway was operative in HC organoids and could be stimulated by low intracellular chloride conditions; (3) forskolin-induced swelling of corrector (VX-809)-pretreated F508del organoids in high chloride vs. high bicarbonate medium was low but could be partially restored by various potentiators in a concentration-dependent manner (CFFT107814, genistein: bicarbonate>chloride at low concentrations, chloride>bicarbonate at high concentrations; VX-770: chloride>bicarbonate at all concentrations tested); (4) the potentiator VX-770 rescued CFTR-dependent, electrogenic HCO₃ secretion in VX-809 pretreated F508del organoids (2D) slightly more efficient than chloride secretion; (5) restoration of CFTR-dependent electrogenic Cl and HCO₃ secretion by VX-770 was transient (lasting <10 min) in F508del- but sustained (>40 min) in S1251N 2D-organoids.

Our preliminary results suggest that, to obtain optimal repair of both Cl and HCO₃ transport, dosing of the CFTR modulators might be critical. Unexpectedly, functional rescue of CFTR activity by VX-770 in intestinal 2D organoids was highly transient in case of F508del-CFTR but sustained in the S1251N gating mutant, reminiscent of the reported VX-770-induced protection from thermal instability of G551D- but not of F508del-CFTR, and in line with the high clinical efficacy of VX-770 in S1251N but not in F508del patients. The protocols developed may facilitate future preclinical testing of novel CFTR repair molecules for their ability to restore bicarbonate transport in organoids from individual CF patients (“personalized medicine”).

Supported by CFF (DEJONG16G0), the Fred Foundation, and FFC#3/2015

Human pluripotent stem cell-derived pancreatic organoids to study cystic fibrosis in a dish

Meike Hohwieler, Anett Illing, Patrick C. Hermann, Justin S. Antony, Qiong Lin, Bruno Sainz Jr., Martin Wagner, Alexander Kleger

Current scientific efforts mainly focus on the pulmonary manifestation of cystic fibrosis (CF) but the pancreatic phenotype represents particularly in long-term survivors an increasingly important hurdle. The precise mechanism how different CFTR-mutations lead to more or less severe pancreatic insufficiency and might influence the development of the pancreas is poorly understood and relevant preclinical models are lacking. Induced pluripotent stem cells (iPSC) present a powerful tool to investigate embryonic development but also to model diseases.

We designed a straightforward approach to recapitulate pancreatic exocrine commitment in the dish using CF patient-specific and control iPSCs generated from plucked human hair-keratinocytes. Briefly, up to 60% of PDX1/NKX6.1-double positive pancreatic progenitors were obtained that could be further differentiated into pancreatic organoids resembling acinar and ductal progeny. Extensive phenotyping of the organoids not only shows the appropriate marker profile but also ultra-structural and functional hallmarks of pancreas. Upon orthotopic transplantation into mice, these organoids form normal pancreatic ducts and acinar tissue resembling human fetal pancreas. We implemented this tool as a model for pancreatic facets of cystic fibrosis (CF) and provide for the first time evidence that pancreatic commitment occurs generally unhindered in CF. There was no significant difference between CFTR-mutated and control iPSCs reaching similar efficiencies of definitive endoderm, pancreatic endoderm and exocrine cells. However in a series of functional assays CFTR-mutated pancreatic organoids mirrored the CF-phenotype: Forskolin-induced activation of the CFTR-channel lead to a pronounced and rapid swelling in wild-type but no relevant reaction in CF patient-derived organoids. We also conducted a scalable proof-of-concept screen using a set of CFTR correctors and activators, and established an mRNA-mediated gene supplementation approach to rescue the functional defect in CF-pancreatic organoids.

Taken together, our platform provides novel opportunities to model pancreatic disease and development *in vitro*. Moreover, we reproduce the CF-phenotype in our organoid system thus allowing patient- and pancreas-specific screening for disease rescuing agents and testing of therapeutic procedures.

Generation of human pluripotent stem cell-derived airway organoids to model cystic fibrosis in vitro

Katherine B. McCauley

Rapid and reproducible derivation of functional airway organoids from human induced pluripotent stem cells (hiPSCs) represents a critical step towards the utility of this model for disease modeling, cell-based therapy and drug screening for airway disorders including asthma, chronic obstructive pulmonary disease, and cystic fibrosis. A limited understanding of the signals regulating human lung patterning during development has made achieving this goal challenging. Using a knock-in NKX2-1 reporter to track and purify early hiPSC-derived respiratory progenitors, we found that Wnt signaling is required for specification of the NKX2-1+ lung epithelium but functions as a key regulator of proximodistal epithelial patterning post-specification. Importantly, we have found that this patterning response is intrinsic to the lung epithelium and is not dependent on signaling from non-lung cell types. We have therefore developed a novel protocol to rapidly and specifically differentiate epithelial-only airway

organoids from hiPSCs. These “bronchospheres” are derived from purified NKX2-1+ lung progenitors, contain key functional airway cell types including secretory, goblet, and basal cells, and can be further expanded and differentiated to multiciliated epithelia in air-liquid interface culture. To provide a proof of principle for the potential clinical utility of this platform, we generated bronchospheres from cystic fibrosis patient-derived hiPSC lines pre- and postcorrection of the $\Delta F508$ mutation in the CFTR gene. These bronchospheres respond in a CFTR-dependent manner to epithelial forskolin swelling assays, highlighting the potential utility of this approach for disease modeling and drug screening for a variety of genetic and acquired airway disorders.

Abstract-Session I: Organoids and CF research funding

CFTR-dependent anion and fluid transport in human bile duct-derived organoid cultures

M.J.C. Bijvelds, M.M.A. Verstegen, K. Burka, H. Roest, M. de Wolf, K.F. Meijssen, H. Gehart, J.N.M. IJzermans, H.R. de Jonge, L.J.W. van der Laan

Introduction: CF is associated with cholestasis and biliary cirrhosis, which is thought to reflect the crucial role of CFTR in ductal bicarbonate and fluid secretion. Presently, we assessed CFTR-mediated anion secretion and fluid transport in bile duct-derived organoid cultures.

Methods: Extra-hepatic bile ducts were collected from donor and explant livers at the time of transplantation. Organoid cultures were initiated from the peribiliary glands (PBGs) and expanded by weekly passaging for >6 months (Huch et al., Cell 2015;160:299-312). Organoids grown in an extracellular matrix (3D culture) were used to assess CFTR-(in)dependent organoid swelling, indicative of secretory fluid transport (Dekkers et al., Nat Med 2013;19:939-45). Anion secretion elicited by cyclic AMP (cAMP)- and calcium (Ca^{2+})-linked (neuro)endocrine factors was assessed in Ussing chambers, using organoid-derived epithelial monolayers grown on permeable supports (2D culture). Gene expression was analyzed by quantitative PCR and mRNA microarray analysis.

Results: In 2D cultures, both cAMP (secretin, VIP)- and Ca^{2+} (ATP, UTP, carbachol)-linked receptor agonists elicited electrogenic anion secretion (8/8 cultures). Cyclic AMP-, but not Ca^{2+} , dependent anion secretion was blocked by pharmacological CFTR inhibition (GlyH-101/CFTRinh172) and by adrenergic α_2 receptor stimulation (UK14,304). VIP and the direct adenylyl cyclase activator forskolin prompted fluid secretion in 3D cultures (3/3), which was attenuated by CFTR blockers and by UK14,304. The muscarinic receptor agonist carbachol also elicited organoid swelling, both in the absence and in the presence of forskolin. In line with these functional data, we found that PBG-derived organoids contain transcripts encoding the epithelial anion channels CFTR and ANO1, and purinergic (P2RY2), muscarinic (CHRM3), secretin (SCTR), VIP (VIPR1), and adrenergic (ADRA2X) receptors.

Conclusion: Organoids originating from the PBGs recapitulate key aspects of anion- and fluid transport regulation in the ductal (cholangiocyte) epithelium. We propose that, in future, patient-derived organoid cultures may be used to model CF-related cholangiopathy.

The effect of silent single-nucleotide polymorphism (sSNP) on CFTR expression

G.B. Bampi, J. Wagner, R. Rauscher, A. S. Ramalho, F. Vermeulen, M.J.C. Bijvelds, H.R. de Jonge, K. De Boeck, Z. Ignatova

Introduction: Synonymous (or silent) nucleotide polymorphisms (sSNPs) are considered neutral at protein level. However, cumulative evidence suggests that sSNPs might also induce phenotypic variability by affecting splicing pattern, translation fidelity, and conformation and function of the encoded protein. A recent publication from our group has shown that T2562G sSNP alters CFTR translation kinetics and consequently the channel stability and function. In this study, we address the effect of another sSNP, c.1584G>A (rs1800095), on the expression and function of CFTR.

Methods: A Belgian patient with mild cystic fibrosis (CF) phenotype lacked any common CF-causing mutations in the first round of screening. Subsequently, the full CFTR gene sequencing revealed a homozygous c.1584G>A sSNP, the only indication as potentially pathogenic. We are applying deep sequencing-based approaches to elucidate the effect of the c.1584G>A mutation at both transcriptional and translational level. As source for the analysis we use patient-derived organoids and compared them to the organoids from Δ F508-CFTR patient and from a healthy individual expressing wild-type CFTR.

Results: We first performed RNA-Seq which reports on transcriptional changes. We first extracted genes differentially expressed in both CFTR-variants and compared them to WT-CFTR (cut-off at +/-2 foldchange, $p < 0.05$). In both CF mutants functional annotation analysis revealed (GO ontology) upregulation of genes involved in cell cycle and RNA repair and downregulation of cilia-associated genes. Furthermore, our analysis focused on capturing G1584A-driven signature comparing G1584A-CFTR expressing organoids to that expressing WT-CFTR. Genes involved in ncRNA processing were upregulated, whereas cell-cycle-related genes were downregulated. The G1584A mutation is also assessed for a role in alternative splicing and exon skipping.

Conclusion: Our preliminary results suggest some significant differences in the gene expression pattern between the CFTR-variants and WT-CFTR at the level of transcription. We are currently performing ribosome profiling to elucidate any causal effects at the level of translation.

Intestinal organoids from genome-edited CFTR iPS cells

Aarne Fleischer, Esther Palomino, Jose M. Martin Fernandez, José Luis Castrillo, Silvia M Ávila-Flores and Ivan M. Lorenzo, Daniel Bachiller

Introduction: Cystic Fibrosis (CF) is a hereditary disease produced by the absence or malfunctioning of the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene. The deletion of the phenylalanine in position 508 (Δ F508) is responsible for more than 70% of the cases described in the European Population. CF is a degenerative disease, which can be considered as the main genetic cause of death in Caucasian children. Unfortunately, different attempts to develop effective gene therapy protocols have not provided satisfactory results. However, stem cell-based regenerative medicine holds big promises for the treatment of many diseases. We therefore propose a novel strategy to develop an efficient treatment for the Cystic Fibrosis disease by combining techniques of gene and cellular therapy in a single protocol.

Methods: The method that we propose includes the following steps: (I) Production of iPS cells from fibroblasts and keratinocytes obtained from Δ F508del CF patients; (II) Correction of the

mutation in the iPS cells; (III) in vitro differentiation of repaired iPS cells towards intestinal organoids; (IV) Evaluation of the functionality of the repaired CFTR gene by using the forskolin induced swelling test.

Results: Here, we present an efficient method for seamless correction of pF508del mutation in patient-specific induced pluripotent stem cells by nuclease-assisted, homologous recombination. Gene correction has been performed in iPS cells from two CF patients by transcription activator-like effector nucleases (TALEN) and a homologous recombination donor vector which contains a PiggyBac transposon-based double selectable marker cassette. CFTR-corrected iPS cells were differentiated into intestinal organoids that were analyzed by the forskolin-induced swelling test. Rapid swelling of mature organoids were observed in cfr-repaired cells confirming the restoration of CFTR function. On the contrary, drug-treated intestinal CFTR mutant organoids did not demonstrate a similar Forskolin-induced swelling capacity.

Conclusion: Here we demonstrate that the combination of iPS cell generation, TALEN-assisted correction of the p.F508del mutation and in vitro cell differentiation is feasible in a single integrated protocol. CFTR gene correction resulted in the restoration of CFTR function in iPSC-derived intestinal organoids.

Intestinal epithelial organoids contribute to supporting drug development and diagnosis

Sara Cald rer, [Anna Baruzzi](#), Silvia Vercellone, Angela Sandri, Luca Rodella, Angelo Cerofolini, Francesco Lombardo, Filippo Catalano, Hugo de Jonge, Baroukh M. Assael, Claudio Sorio, Paola Melotti

Introduction: In vivo and ex vivo measurements of CFTR function in human cells and tissues can be used for screening and monitoring new therapies and phenotyping of controversial CFTR genotypes. Among the tools currently available, a technique enabling intestinal stem cells to expand into closed organoids containing crypt-like structures and an internal lumen lined by differentiated cells, recapitulating the in vivo tissue architecture (Sato et al Gastroenterology 2011) was set up in our lab and used for measuring CFTR function by a simple and quantitative assay.

Methods: We successfully developed intestinal organoids from 12 of 14 non-CF (in 4 cases n>3 vials have been stored); 19 of 27 CF of which n>3 vials have been stored for 8 cases; and 7 from 8 CRD (CFTR related disease) subjects. We then measured forskolin induced swelling (FIS) (Dekkers et al Nat Med, 2013) before and after drugs exposure to VX809, VX770 and Ataluren.

Results: In non-CF organoids swelling was completely blocked by the CFTR (inh)-172 and significantly enhanced following treatment with the potentiator Ivacaftor (VX770). Swelling rates in CF organoids were significantly different than in non CF and variable among CF patients, very likely dependent on CFTR mutations. Remarkably, in organoids from a CF patient carrying the R553X / 2789+5G>A CFTR genotype we observed swelling following exposure for 24h to the premature termination corrector Ataluren (PTC124), but not in its absence. Moreover beneficial effects of Ataluren in vivo in this patient were detected by improvement of lung function (FEV1), and nasal potential difference (NPD) during clinical trials PTC124-GD-009-CF, PTC124-GD-009e-CF and PTC124-GD-023-CF. Swelling was improved after treatment with Ataluren in intestinal organoids of another CF patient (W1282X/R117H, IVS8:T7/T7) while no changes were detected in other organoids with nonsense CFTR mutations. The presence of nonsense CFTR transcripts was indirectly investigated by swelling induction following treatment with G418. We detected

improvements of CFTR-associated function using correctors and potentiators as VX809 and VX770, respectively in other selected genotypes.

Conclusion: We have been able to grow intestinal organoids from CF and non-CF subjects to evaluate CFTR function by FIS assays showing partial restoration of CFTR activity in response to CFTR potentiators and CFTR correctors. In a case we detected recovered CFTR expression and function following treatment with Ataluren in intestinal organoids matching a clinical improvement. This study reinforces the need to personalize therapy in CF, confirming intestinal organoids as a suitable tool for this purpose.

This work was supported by the Italian Cystic Fibrosis Research Foundation (FFC grant#7/2016: Delegazione FFC Belluno) and by the Lega Italiana Fibrosi Cistica Associazione Veneto Onlus.

HIT CF: a successful research program on personalised medicine through collaboration with patients, clinicians and researchers

Vincent A.M. Gulmans, Cornelis K. van der Ent, Jeffrey M. Beekman, Ineke Braakman, Bob S. Scholte, Hugo R. de Jonge, Jacquelin, J. Noordhoek

Introduction: A close and effective collaboration between clinicians, researchers and patients representatives should ultimately lead to a research program that is targeting the needs of patients, as prioritized by themselves. The role of the patients representatives in developing, executing and funding the program is crucial.

Methods: Through close cooperation between the Dutch Cystic Fibrosis Foundation (NCFS), clinicians and researchers, a scientific research program was defined in 2012, called "HIT CF". It consisted of 4 tracks with 16 projects, with a budget summing up to € 4.3 million. The HIT CF program aimed to establish a breakthrough in CF treatment through further development of compounds that effectively modify basic disease mechanisms, combined with innovative ways to test drug efficacy with organoids in individual patients. Another part of the program consisted of projects on inflammation and infection.

Results: The programme has led to a laboratory setting in which the efficacy of CFTR modulators can be predicted in organoids of individual patients, which will enable precision medicine by targeting the treatment (or combination of treatments) to the most responsive patients. HIT CF 1 has been running for 4 years and has led to more than 40 publications. 400 patients were involved and 30 researchers. Off- label treatment and reimbursement of CFTR modulators has been established already for the first 7 patients, with much more more to follow. Results of a nation wide survey showed that 97% of the patients and parents wished a continuation of the research program, but completely focussed on personalised medicine.

Conclusion: Tight collaboration between between clinicians, researchers and patients representatives in a research program has proven to be very successful. This resulted in a recently launched 5 years HIT CF 2.0 program with a budget of € 5.4 million, with extension to a European level.

Keynote Session

Intestinal organoids, fluid secretion and cystic fibrosis: the past, present and future

Jeffrey Beekman

New stem cell culture technologies have enabled the culture of miniature organs in vitro. One such approach makes use of somatic or adult epithelial stem cells that grow into 3D structures termed organoids. Intestinal organoids derived from rectal biopsies can be used to study CFTR function by measurement of organoid swell phenotypes, either under standard culture conditions or induced by forskolin.

This presentation will provide an overview of the use of organoids for CF drug discovery and patient stratification. The performance of this biomarker of CFTR function is compared to other biomarkers of CFTR function such as SCC and ICM, in the context of disease severity stratification and typing of individual CFTR modulator efficacy. New organoid based models from various tissues including lungs and pancreas will be discussed as well as ongoing studies and the impact of this model on future personalized care.

Sessions II: Impact for CF

Organoids for use in clinical trials

Kris De Boeck

Up till now, the forskolin induced swelling assay (FIS) in intestinal organoids has the best track record as *ex vivo* biomarker of CFTR function for use in precision medicine in patients CF. Thanks to the excellent team work of the Dutch basic scientists, clinicians and patient organization we know that: mean lung function results in clinical trials with CFTR modulators correlate very well with mean *ex vivo* results in intestinal organoids from patients with these same *CFTR* mutations; for patients with rare *CFTR* mutations FIS results are a good predictor of clinical benefit (positive example G1249R and negative example G970R); FIS results differ between patients with the same mutation e.g. F508del homozygous subjects; high FIS responders remain high responders; subjects with a high FIS response have on average a larger clinical benefit than low responders.

Also in our lab we have been able to confirm the very good correlation between clinical trial results of CFTR modulators in subjects with specific mutations and FIS results in organoids derived from patients with similar genotypes. The correlation even improves when only the swelling induced by modulators is considered, i.e. after subtraction of the forskolin part. The correlation between clinical trial data and FIS results thereby approaches and even equals the correlation between clinical trial data and results in human bronchial epithelial cells, the current gold standard biomarker. We thus feel confident that organoids response *ex vivo* can be used as a biomarker to select patients for inclusion in clinical trials.

At present, we have a promising treatment pipeline (triple combo) for subjects with at least one F508del mutation, i.e. around 85% of patients with CF. But that also means that we do not have a treatment for the remaining 15% of patients, especially those with rare or ultrarare *CFTR* mutations.

For clinical trial methodology, the oncology field has led the way: basket trials and platform trials have been introduced. Using organoids as biomarker, these new trial types are also ideal for application in CF. In a basket trial, patients with different *CFTR* mutations but who have a

positive organoid response to a specific modulator/combination of modulators *ex vivo* can be grouped in one treatment arm. The treatment benefit in these patients is then compared to that in subjects who have a negative FIS response to the same CFTR modulator. That way we not only offer treatment to selected patients but we also further build the evidence of the validity of organoids as a biomarker.

In the longer term, when several CFTR modulators have become available, we can consider a 'platform trial'. Indeed, in this method, new CFTR modulators can be evaluated in a continuous way, using the same standardized clinical trial protocol (e.g. cross-over trial) with organoids as the biomarker to assign patients to either treatment arm (biomarker positive versus negative). Treatment with the new modulator can then be compared to the current standard of care. A platform trial also allows for other biomarkers to come in as time goes on. Trial arms can be stopped because of success or because of futility if needed.

Within the Horizon 2020 sponsored European plan, we will identify patients with rare *CFTR* mutations using data from the European CF registry and invite them to have intestinal biopsies taken to grow organoids. We will fully standardize the organoids FIS assay between European sites and determine FIS responses in patients with rare or ultrarare *CFTR* mutations. This will then culminate in clinical trials with new *CFTR* modulators or modulator combinations in responsive versus non-responsive patients. The entire program will have participation of CF Europe and the European Cystic Fibrosis Clinical Trial Network.

CFF Perspective on Ex Vivo Models as Tools for Predicting CFTR Modulator Responses

Katherine L. Tuggle

Cystic fibrosis (CF) is a rare genetic disease caused by more than 1,700 mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*), an anion channel that regulates ion and fluid transport in epithelial tissues and organs. In recent years, *CFTR* modulators have been developed that target the underlying defect in the dysfunctional *CFTR* protein. However, these drugs are only available to a limited number of patients who may benefit based on current regulatory processes. In the U.S., approximately 40% of CF patients have genotypes that are not currently treated by approved therapies, while laboratory data suggests that some of these mutations may be responsive to current *CFTR* modulating drugs. Human bronchial epithelial (HBE) cells, derived from explanted lungs, have served as a backbone for evaluating effectiveness of *CFTR* modulator drugs as part of discovery and development efforts, and have been an essential part of the drug approval process. Difficulty in acquiring HBE cells with a wide range of mutations has limited the ability to study effects of these drugs on rare *CFTR* mutations thereby presenting a significant challenge for expanding therapies to treat additional genotypes. In order to ensure all people with responsive mutations are able to benefit from these life altering therapies, the Cystic Fibrosis Foundation (CFF) has engaged researchers around the world to develop promising new tools and models, including laboratory and patient derived cell-based systems, that have the potential to

- 1) identify drug responsiveness of rare *CFTR* mutations,
- 2) tailor treatments for personalized medicine,
- 3) aid drug discovery and early drug development programs, and
- 4) influence regulatory pathways.

Two of the models in development include human nasal epithelial (HNE) cells and intestinal organoids. Like HBE cells, these models are collected from disease relevant tissues, however,

they provide additional advantages including the ability to collect rare genotypes from targeted populations and the potential for repeated sampling. This presentation will focus on cell-based *in vitro* models being developed to understand and predict CFTR modulator responses of rare mutations as part of our strategy for ensuring all patients who will benefit from approved and marketed modulators can access the drugs.

Abstract Sessions II: Basic CF defect and pharmacological correction

Bicarbonate and chloride transport assays using fluorescent biosensor cell lines for identification of novel synthetic anion transporters

Kevin Bernhard, Cordula Stahl, [Manfred Frey](#)

Introduction: The cystic fibrosis transmembrane regulator protein (CFTR) is a driver of chloride-bicarbonate exchange across the membrane, defining the salt and water contents and the acidity of the periciliary fluid. Failure of bicarbonate transport will reduce the periciliary fluid and will produce thick mucus, facilitating chronic infections in the airways. The measurement of intracellular bicarbonate concentration changes in living cells by a fluorescent sensor protein would be helpful in measuring bicarbonate transport processes.

Methods: A cell line expressing a recombinant bicarbonate sensor and reporter protein was developed. This cell line was used in establishing cell based assays allowing the measurement of intracellular bicarbonate concentration changes. A chloride biosensor cell line and a cell based assay were developed. Bicarbonate and chloride influx assays were performed in a microplate reader format.

Results: Bicarbonate and chloride transport activities were measured for several synthetic anion transporters (anionophores).

Conclusion: The developed cell based bicarbonate and chloride transport assays can be used for the screening of new compounds which could recover the anion transport in CFTR defect cells.

Setup of a simplified method to measure CFTR-dependent iodine transport: HS-YFP assay

Monica Aversa, Silvia Vercellone, Marco Pedrazzi, Margherita Bavestrello, Laura Minicucci, Federico Cresta, Rosaria Casciaro, Sara Caldrer, Federica Quiri, Hugo De Jonge, Edon Melloni, Anna Baruzzi, Baroukh M. Assael, Paola Melotti, [Claudio Sorio](#)

Introduction: In order to measure in minimal invasive manner CFTR activity, we set up a method to assay functional CFTR and focused on leukocytes from healthy and CF donor. Leukocytes are recognized in the scientific literature as key component of the pathogenetic events associated to cystic fibrosis and represent an easily accessible source of primary cells that might be exploited to monitor CFTR expression and activity.

Methods: The readout of this method is based on residue quantity of iodine present in the cell supernatant, after proper stimulation, quantified by the fluorescence of Halide Sensitivity Yellow Fluorescence Protein (HS-YFP).

Results: The specificity of the assay for CFTR activity is tested using two different CFTR inhibitor, CFTR-172 and PPQ-102, and downregulating CFTR (siRNA technology) in acute monocytic leukemia MM6 cell line, highly expressing CFTR. These data were confirmed also in peripheral

blood leukocytes, collected by venipuncture (3-5 mL) performed in different centers and by different operators. Significant differences between WT and CF peripheral blood mononuclear cells (PBMC) and purified monocytes were recorded. Of note are the results obtained from HS-YFP assay performed in PBMCs of a patient carrying the G1349D/F508del mutation and taking Ivacaftor in which we have measured a significant increase in iodine transport in all the time points after the start of the treatment (7 time points starting from October 2015 to September 2016).

Conclusion: HS-YFP assay might represent a minimally invasive, convenient and fast method to measure CFTR function in leukocytes. Monitoring CF patients taking CFTR correctors or potentiators with a simple blood test can represent a further step toward a personalized medicine approach in CF.

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Testing CFTR function in vivo by imaged ratiometric measurement of beta adrenergic/cholinergic sweat rate in human sweat glands

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Introduction: Increasing interest in personalized medicine approach in cystic fibrosis (CF) focuses on cystic fibrosis transmembrane conductance regulator (CFTR) targeted therapies. Detecting CFTR improvement in single patients requires standardized outcomes for CFTR function in vivo. A bioassay was reported by Wine et al. (PlosONE 2013, 2017) for measuring sweat rates in individual human sweat glands. We aimed to adapt the published procedure in order to obtain a simplified set up, procedure and image analysis, commercial availability of instruments, reproducible results and cost reduction.

Methods: Sweat secretion rates were given by changes of volume of sweat drops secreted on the forearm in an oil layer, including the presence of a water-soluble blue dye (erionin disodium crystals). We computed a ratio between CFTR-dependent, evoked by intradermal microinjection of a β -adrenergic cocktail (C-sweat), and CFTR-independent, induced by methacoline as cholinergic stimulus (M-sweat), sweat secretion rates by multiple individual glands. The analysis was performed in 22 CF patients, 22 non-CF subjects (CTR), 22 healthy carriers (HTZ) and 3 clinical cases.

Results: We obtained an approximately linear readout of CFTR function: the carriers mean ratio was 0.51 the value of non-CF controls while the average ratio of CF subjects was around zero. In a patient affected by CFTR related disorder we found a value in between CF and HTZ mean values. All groups were clearly discriminated with extremely significant differences of C-sweat/M-sweat ratios ($p < 0.0001$ for three groups comparison). This method discriminates between CF and non-CF patients (non-CF controls and heterozygotes), providing sensibility and specificity of 100%. It discriminates between heterozygotes and non-CF controls, providing sensibility 82% of specificity of 86%. We obtained reproducible discrimination when different operators performed the test. A software was developed for detecting sweat bubbles, paving

the way for automatically mapping and measuring sweat bubbles as required for automated image analysis.

Conclusion: This bioassay is capable to clearly discriminate among non CF, healthy carriers and CF individuals at variance with Gibson and Cooke gold standard sweat chloride assay, is minimally invasive and thanks to its exquisite sensitivity and specificity appears suitable for multicentre studies focusing on CFTR targeted therapies and to assist in the diagnosis of controversial cases. This approach can simplify the analysis and thus promote a better understanding of the functional relevance of rare CFTR mutations.

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Abstract Sessions III: Basic CF defect and infectiology

Clarithromycin-loaded nanoparticles to treat *M. abscessus* lung infection

Adriely Góes, Cristiane Carvalho-Wodarz, Claus-Michael Lehr

Introduction: The treatment of intracellular lung infections caused by non-tuberculous mycobacteria (NTM) is challenging due to their resistance to most of the antibiotics. *Mycobacterium abscessus* is the most resistant NTM isolated from lungs of cystic fibrosis patients. The two different morphotypes of *M. abscessus*, smooth (S) or rough (R), are associated to diverse disease severity, in which the R variant is responsible for a more aggressive infection. One of the antibiotics recommended for the treatment of mycobacterial infection is clarithromycin (Clari), a macrolide that presents also anti-inflammatory activity. However, Clari has poor solubility and low bioavailability, which has contributed to the resistance development in *M. abscessus*. Therefore, our hypothesis is that these problems can be overcome by designing nanocarriers for improving the intracellular delivery of anti-infectives.

Methods: In the present study, we have encapsulated Clari in chitosan-coated poly (lactid-co-glycolid acid) nanoparticles (Clari-NPs), suitable for aerosol delivery by nebulization of an aqueous dispersion. Clari-NPs were then used against intracellular *Mycobacterium abscessus* (S and R variants), in either A549 or RAW 264.7 cells. Intracellular bacteria survival was determined by colony forming unity (CFU).

Results: Clari-NPs were effective against both variants of *M. abscessus* in RAW cells, with a greater activity against S variant (80% killing rate) comparing to the free Clari (60% killing rate). Clari-NPs were also effective against *M. abscessus* internalized by A549 cells.

Conclusion: Overall, these data revealed that Clari-NPs are highly efficient against both variants of *M. abscessus* intracellular.

Modelling cystic fibrosis infected airways with a 3D in vitro model of human bronchial epithelia and macrophages to evaluate anti-infective formulations

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Introduction: To reach bacteria in the airway, inhaled medicine should overcome biological barriers (e.g. epithelium, mucus, and immune cells). Therefore, innovative formulations and in vitro systems are of great need in the development of novel anti-infective aerosol medicines. Aiming for predictive in vitro systems that mimic cystic fibrosis infected airways, we established two new models, both infected with *Pseudomonas aeruginosa*: model A) bronchial epithelium plus human mucus and pre-formed *P. aeruginosa* biofilm; model B) bronchial epithelium plus human macrophages and planktonic *P. aeruginosa*. Using the first model, we tested the efficacy of novel aerosolized ciprofloxacin-loaded nanocarriers [1] against *P. aeruginosa* biofilm.

Methods: CFBE410- cells, a human cystic fibrosis cell-line, grown at the air-liquid interface (ALI) (in a 0.4 µm insert), was covered with human tracheal mucus (collected by endotracheal tube methods) [2] and infected with a pre-formed biofilm of *P. aeruginosa* PAO1. Ciprofloxacin-loaded nanocarriers were used as anti-infective formulation and bacterial survival determined via CFU. Cell viability and epithelial barrier integrity was determined via MTT and TEER, respectively. Model B) Macrophages (THP-1 differentiated cells) were added to CFBE410- cells at ALI (in a 3.0 µm insert), and infected with planktonic bacteria.

Results: To investigate the efficacy of novel inhaled medicine, CFBE410- cells were incubated with human mucus, and further infected with pre-formed *P. aeruginosa* biofilm. When applied 1 h after the infection, ciprofloxacin-loaded nanocarriers were able to significantly reduce the number of viable bacteria, either in the planktonic fraction (log 6 reduction) or in the biofilm fraction (log 10 reduction), while bronchial epithelial cell viability and barrier properties were maintained. To investigate biofilm formation and macrophage behavior the model B was used. Macrophage transmigration was detected 3 to 6 hours after infection and, at 6 hours, there was indication of early bacteria aggregation to form biofilm.

Conclusion: With application of pre-formed biofilms of *P. aeruginosa* on monolayers of bronchial epithelial cells, we set up an experimental protocol with an appropriate time window to test aerosolised formulations against the pathogen. The integration of macrophages in the infected co-culture allowed to assess macrophages role in an infected epithelial co-culture along the transition to biofilm, and will be further explored to assess inflammatory response and efficacy of novel inhaled medicines.

[1] Türeli et al Eur J Pharm Biopharm. 2017 May 2

[2] Murgia et al Eur J Pharm Biopharm. 2017 Apr 1

Evaluation of the primary human nasal epithelial cell cultures as a biomarker in personalized Cystic Fibrosis treatment

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Introduction: Clinical studies with CFTR modulators have demonstrated that functional restoration of the mutated CFTR can be translated into benefit for patients. However, high variability of patients' responses highlights the importance of finding relevant biomarkers to

predict the clinical responses. Primary human nasal epithelial (HNE) cells are easy to collect by nasal brushing and allow quantification of cAMP-mediated chloride transport as an indicator of CFTR function.

Methods: CFTR activity in vitro was assessed in primary HNE cultures, in comparison to human bronchial epithelial cells, by short circuit-current (Isc) measurements. Variation of Isc after application of Forskolin/IBMX and CFTR potentiator VX-770 (Δ IscF/I+V in μ A/cm²) was an index of the CFTR-dependent chloride secretion. CFTR function in vivo was assessed via nasal potential difference (NPD) measurements in patients. CFTR expression in the apical surface of reconstituted epithelia was assessed by CFTR immunostaining and semi-quantification of the apical fluorescence.

Results: Isc measurements in HNE cultures recapitulated the results obtained in HBE cultures from CF patients and healthy controls, and were accurate enough to distinguish different levels of CFTR function. CFTR activity in HNE cultures from F508del homozygous patients (Δ IscF/I+V=0.2 (0.1)) was significantly lower than that found in patients with genotypes associated with a residual CFTR function or a wide spectrum genotype (Δ IscF/I+V=8.8 (7)). In cultures from F508del healthy carriers, CFTR activity was significantly lower than that of healthy donors (Δ IscF/I+V= 5.1 (1.4) versus 11.6 (2.6); p=0.03). These results were correlated to apical CFTR expression (R²=0.9, p< 0.0001) and in vivo CFTR activity as measured by nasal potential difference (R²=0.82, p< 0.0001).

We then evaluated the correction of CFTR function by VX-809 in HNE cultures issued from patients homozygous for the F508del mutation or carrying CFTR genotypes displaying a wide spectrum of CFTR activity. VX-809 treatment of F508del homozygous cells significantly increased the average Δ IscF/I+V as compared to DMSO by a mean of 1.8 μ A/cm² (0.6-3.1), reaching an average of 25% (1-69%) of the WT-CFTR level (p<10⁻⁶). This change was correlated to an increase in the CFTR apical expression (R²=0.6, p<0.001). Finally, we assessed the predictive value of the primary HNE cell cultures by comparing the in vitro pharmacological rescue by CFTR modulators to the clinical efficacy of this treatment in patients. In vitro correction levels were significantly correlated to respiratory function improvement in 8 F508del homozygous patients who had initiated the combination of VX-809 and VX-770 (Orkambi®) treatment. Patients whose FEV1 improved by more than 5% displayed a mean change of Fsk/IBMX+VX-770 response upon 10% of the average WT level.

Conclusion: We provide the first evidence that correction of CFTR function and expression in HNE cell cultures can reliably predict respiratory improvement in patients to be treated with CFTR modulators. Therefore, it may be used as a surrogate biomarker to preselect responder patients for personalized therapy.

Session III: Perspectives

Induced pluripotent stem cells for identification of novel drug combinations targeting cystic fibrosis lung and liver disease

Ulrich Martin

Effective drugs are still not available for the treatment of most Cystic Fibrosis (CF) patients, and associated lung and liver pathologies remain untreatable. As a result, there is clearly an unmet need for novel correctors of F508del-CFTR, and in particular for other rare trafficking (class II)

and nonsense (class I) mutants. Novel compounds have to be identified, and tailored in combination to specific CFTR mutations, to different tissues, or even to the individual patient.

Immortalized cell lines overexpressing mutant CFTR are typically used to screen candidate molecules but have proven to be poor predictors of clinical efficacy. The complexity of CFTR maturation and turnover requires the use of cellular models that closely recapitulate the specific properties of the clinically most affected organs. Importantly, current screening efforts based on primary airway cells or intestinal organoids cannot specifically target single rare CFTR mutations, or mimic multiple cell types.

With the major breakthrough presented by induced pluripotent stem cells (iPSCs), another patient and disease-specific cell source for disease modelling and drug screening is now on hand, that can be easily obtained from any patient. Major advantages of iPSCs include the possibility to be genetically engineered on a clonal basis using novel genome engineering technologies, to be expanded to large cell numbers and to be specifically differentiated into different CF disease-relevant lineages, in particular respiratory epithelium.

The presentation will provide an overview on the current state of the art concerning the development of iPSC-based personalized surrogate models of CF lung and liver disease and use of iPSCs for CF drug screening.

Multiple impacts of rare mutations on CFTR function

David N. Sheppard

The common life-limiting genetic disease cystic fibrosis (CF) is caused by mutations in the ATP-binding cassette (ABC) transporter cystic fibrosis transmembrane conductance regulator (CFTR; ABCC7), which plays a pivotal role in fluid and electrolyte transport across epithelia. To date, more than 2,000 mutations have been identified in the CFTR gene. Studies of the molecular mechanisms of CFTR dysfunction in CF have identified six different classes of mutation, which disrupt either the production of CFTR protein, its delivery to, and stability at, the plasma membrane or function as a regulated Cl⁻ channel. Some CF mutations cause CFTR dysfunction by one mechanism only. For example, G551D, the first mutation to be rescued by a clinically-approved CFTR modulator (the potentiator ivacaftor) is a class III mutation, which disrupts channel gating only. However, many CF mutations, quite likely the majority, cause CFTR dysfunction by multiple mechanisms. This is best illustrated by F508del, the most common CF mutation, a class II-III-VI mutation, which disrupts the processing of CFTR protein, its plasma membrane stability and regulation by phosphorylation and intracellular ATP. Using high-resolution single-channel recording data, this presentation will discuss how different CF mutations disrupt CFTR channel gating and explore their responses to small molecule CFTR modulators. It will highlight the spectrum of impacts of different mutations on CFTR channel gating, showing how some mutations have subtle effects, while others have severe consequences.

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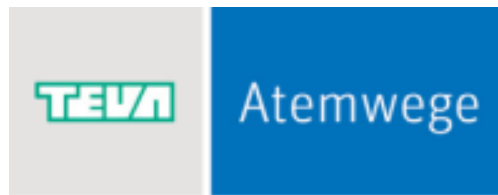
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