MUKOVISZIDOSE
Mukoviszidose Institut gGmbH

19th Scientific Meeting

Future medicine in CF

19th – 20th September 2019

Schloss Montabaur (Germany)

Organization

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

Chairs

Andreas Hector (CH/Zürich)

Mirjam Stahl (DE/Heidelberg)

Burkhard Tümmler (DE/Hannover)

Table of content

Please not that not all talks have a published abstract.

Program

Thursday, September 19th 2019

11:30-12:10 pm	*Get together + Lunch*
12:10-12:20 pm	**Opening of the meeting + short introduction**
	Manfred Ballmann (Chairman of the supervisory board Mukoviszidose Institute) + Chairs
12:20-2:20 pm	**Session I**
	Moderation: Andreas Hector (CH/Zürich)
12:20-1:00 pm	**Joseph Rosenecker (DE/Bad Hindelang)** Self-assembled peptide-poloxamine nanoparticles enable in vivo genome restoration of the CFTR-gene in murine airways of CF mice
1:00-1:40 pm	**Antje Munder (DE/Hannover)** Cell-based therapies to treat chronic CF Lung Disease - Data from mouse models
1:40-2:20 pm	**Christine Rohde (DE/Braunschweig)** Bacteriophage therapy for patients with chronic bacterial lung colonization
2:20-2:40 pm	*Coffee break + free discussion*
2:40-4:00 pm	**Session II**
	Moderation: Anna-Maria Dittrich (DE/Hannover)
2:40-3:20 pm	**Jeffrey Beekman (NL/Utrecht)** Organoids for treatment of rare mutations
3:20-4:00 pm	**Isabelle Sermet-Gaudelus (FR/Paris)** Concepts for future patient care in CF
4:00-4:30 pm	*Coffee break + free discussion*
4:30-5:40 pm	**Session III**
	Moderation: Carsten Schwarz (DE/Berlin)
4:30-4:45 pm	**Constanze Vitzthum (DE/Berlin)** – *submitted abstract* Comparison of efficacy of functional rescue of F508del CFTR by approved CFTR modulators
4:45-5:25 pm	**Lutz Nährlich (DE/Gießen)** Patient registry and implications for phase IV trials
5:25-5:40 pm	**Wolfgang Bernhard (DE/Tübingen)** – *submitted abstract* Choline supplementation to treat severe hepatosteatosis in a compound heterocygous CF patient
5:40-6:00 pm	*Coffee break + free discussion*
6:00-7:00 pm	**Keynote-Session**
	Moderation: Burkhard Tümmler (DE/Hannover)
	Paul Eckford (CA/Toronto) The CF Canada-Sick Kids Program in individual CF therapy: Towards personalized medicine in CF
8:00 pm	*Dinner (Hotel Schlemmer)*

Friday, September 20th 2019

8:00-9:55 am	**Session IV** *Moderation: Barbara Kahl (DE/Münster)*
8:00-8:50 am	**Mirjam Stahl (DE/Heidelberg) + Yin Yu (DE/Heidelberg)** Suitable clinical biomarkers for safety and longterm efficacy evaluation and current possibilities
8:50-9:10 am	**Sébastien Boutin (DE/Heidelberg) + Simon Gräber (DE/Berlin)** – *submitted abstracts* Effects of Lumacaftor-Ivacaftor therapy on lung microbiome and lung disease detected by magnetic resonance imaging in F508DEL homozygous patients with Cystic Fibrosis
9:10-9:25 am	**Dario Frey (DE/Heidelberg**) – *submitted abstract* Relationship between microbiome dysbiosis, inflammation neutrophil recruitment and lung function in CF patients
9:25-9:40 am	**Justus Horstmann (DE/Saarbrücken)** – *submitted abstract* CF bronchial epithelial cells infected with *P. aeruginosa* biofilm at air-liquid interface for new anti-infective test
9:40-9:55 am	**Hanna Schmidt (DE/Ulm)** – *submitted abstract (canceled)* Effect of hyperosmotic solutions on ion and water transport properties of airway epithelia
09:55-10:20 am	*Coffee break + free discussion*
10:20-11:40 am	**Session V** *Moderation: Manfred Ballmann (DE/Rostock)*
10:20-11:00 am	**Anna-Maria Dittrich (DE/Hannover)** Improving adherence by digital support - The transfer CF pilot trial
11:00-11:40 am	**Ed Nash (UK/Birmingham)** Home Monitoring in adults with CF
11:40-12:10 pm	*Coffee break + free discussion*
12:10-1:30 pm	**Session VI** *Moderation: Mirjam Stahl (DE/Heidelberg)*
12:10-12:50 pm	**Jim Wild (UK/Sheffield)** Functional and structural lung MRI to detect early abnormalities and change in cystic fibrosis
12:50-1:30 pm	**David Sheppard (UK/Bristol)** Mechanism of CFTR dysfunction and modulator responsiveness of cystic fibrosis variants that replace the same residue by distinct amino acids
1:30 pm	**Closing of the Meeting** Chairs
1:40 pm	*Lunch*

Session I

Self-assembled peptide-poloxamine nanoparticles enable in vivo genome restoration of the CFTR-gene in murine airways of CF mice

Joseph Rosenecker

The small molecule approach has resulted in three drugs which are currently licensed for 34 of the 281 in vitro characterized CF-causing mutations. There is a strong pipeline of "next generation" small molecule drugs determined by high throughput which are widely predicted to be able to treat the disease effectively in ~90% of people with CF. However, approximately 10% of the CF population (roughly 8,000 individuals) have a combination of mutations which disrupt mRNA splicing and/or create a PTC (premature termination codon) who will not benefit from this approach. One possibility for these individuals bearing those "non-rescuable" mutations is the CFTR cDNA-based gene therapy approach which in principle can genetically complement and functionally correct essentially all CF-causing mutations. However, after more than two decades of extensive research and more than 20 clinical trials, no substantial clinical benefit has been reported. An alternative to cDNA addition is CFTR-gene integration into the genome of the affected airway epithelial cells for protein replacement. Over the last seven years, we have been developing a technique for in vivo genome restoration using sleeping beauty mediated transposition in cystic fibrosis mice (Guan et al. 2019). The gene carrier system which was employed by our group is based on poloxamine-based copolymers. Unlike conventional cationic polymers or lipids, the emerging poloxamine-based copolymers display promising in vivo gene delivery capabilities. However, poloxamines are invalid for in vitro applications and their in vivo transfection efficiency is still low compared with viral vectors. Here, we show that peptides developed by modular design approaches can spontaneously form compact and monodisperse nanoparticles with poloxamines and nucleic acids via self-assembly. Both mRNA and plasmid DNA expression mediated by peptide-poloxamine nanoparticles are greatly boosted in vitro with negligible toxicity. In the next step, peptide–poloxamine nanoparticles were evaluated in vivo for the delivery of the Sleeping Beauty (SB) transposon system consisting of SB transposon encoding CFTR (pDNA) and transposase-encoding SB100X-mRNA, as a transient source of transposase to avoid potential genome-toxic transposon reintegration. mRNA and pDNA delivery by peptide–poloxamine nanoparticles was performed by intratracheal application in cystic fibrosis mice with three-times repeated dosings. Our results revealed consistent long-term CFTR expression on the apical side of the superficial epithelium of bronchial airways with a safe integration profile. The platform technology developed in the current study holds potential for clinical applications CF patients in the future.

Self-assembled peptide-poloxamine nanoparticles enable in vitro and in vivo genome restoration for cystic fibrosis. Guan S, Munder A, Hedtfeld S, Braubach P, Glage S, Zhang L, Lienenklaus S, Schultze A, Hasenpusch G, Garrels W, Stanke F, Miskey C, Johler SM, Kumar Y, Tümmler B, Rudolph C, Ivics Z, Rosenecker J.
Nat Nanotechnol. 2019 Mar;14(3):287-297. doi: 10.1038/s41565-018-0358-x. Epub 2019 Jan 28.

Cell-based therapies to treat chronic CF Lung Disease - Data from mouse models

Antje Munder

Introduction: Major achievements in treatment of cystic fibrosis (CF) have been made through development of mutation-specific therapeutics. Nevertheless, implications of this monogenetic disease are still fatal and the defective CFTR channel in epithelial cells cannot sufficiently explain the severe chronic inflammation and exacerbations driven by bacterial infections in the lungs of CF patients. In our studies, we therefore focus on the role of immune cells like macrophages in CF lung disease and use pulmonary macrophage transplantation to combat experimental *Pseudomonas aeruginosa* (*P. aeruginosa*) airway infection in mice.
Methods: We transplanted stem cells from the bone marrow of healthy mice into CF mice and investigated the outcome of transplantation in a standardized acute *P. aeruginosa* airway infection. Engraftment of transplanted cells was tracked via flow cytometry and immune fluorescence microscopy. Furthermore, we used induced human pluripotent stem cells (iPSCs) to generate iPSC-derived macrophages (iPSC-MAC). These CD45+CD11b+CD14+CD163+ macrophages were produced in industry-compatible, stirred-tank bioreactors. Functionality of iPSC-MAC was assessed in immunocompromised mice infected with *P. aeruginosa*.
Results: CF mice transplanted with healthy wild type cells displayed better survival, reduced lung bacterial numbers and a milder disease course in total. Tracking of transplanted cells revealed their successful engraftment in murine lungs and their major differentiation into cells with macrophage characteristics. Bioreactor-derived iPSC-MACs rescued mice from *P. aeruginosa* mediated airway infection within 4-8 hours post intra-pulmonary transplantation and reduced bacterial load more than 100-fold.
Conclusion: We assess our findings as highly important in providing insight into the role of macrophages in CF lung disease. Generation of macrophages from iPSC-sources in scalable stirred-tank bioreactors and pulmonary transplantation of iPSC-MACs may allow further innovative cell-based treatment strategies for CF and other patients suffering from bacterial lung infections.

Bacteriophage therapy for patients with chronic bacterial lung colonization

Christine Rohde

Introduction: Bacteriophages (in short: phages) have been successfully used in antibacterial therapy since about 100 years in countries of the former Sovjet Union and to a much lesser extent also in the West. To counteract the growing antibiotic multi-drug resistance (AMR) crisis and to reduce the manifold AMR-generated consequences, phage therapy offers an attractive additional avenue in human medicine to address the problem. Lytic phages are the natural enemies of bacteria and by affecting only strains within a species, microbiome dysbiosis caused by tailored specific phage action is very unlikely. Toxic, allergenic, mutagenic or other side effects or lytic interaction with eukaryotic cells are not known and bacterial phage resistance does not create sustainable problems. However, phage preparations for medical use must be purified according to pharmaceutical standards and national regulations and the phage entities must be well characterized on biological level. Only on this basis, licensing of single phage preparations and a model licensing pathway according to western standard can become real. It requires more preclinical research and systematic clinical trials. For aiming at reduction of chronic bacterial lung colonization by phage application, the DSMZ phage working group initially worked on the phage-host biology of a typical bacterial colonizer, *Achromobacter xylosoxidans* in an in vitro study, participated in a preclinical pilot study involving an in vivo mouse lung model with *Acinetobacter baumannii* and is a partner in the consortional project Phage4Cure, the first German project aiming at a clinical trial involving phage therapy. Clinical partner in both a. m. approaches is the Charité University Hospital Berlin, Department of Infectious Diseases and Respiratory Medicine. All DSMZ phage work has been in vitro on microbiology laboratory level.
Methods: All phages used in the in vitro study, in the preclinical pilot study or in the project Phage4Cure have been characterized on biological level, as appropriate. Bacterial host strains were received from different culture collections or from clinical collections with diverse origins, they were characterized with respect to species identity, antibiotic resistance spectra and virulence markers to evaluate phage specificity and efficiency. The preclinical pilot study aimed at determining efficiency, safety and tolerability of a purified phage preparation: Mice were transnasally infected with *A. baumannii* and treated with phages intratracheally. Bacterial burden in lung, BALF, blood and spleen were analyzed p. i., leukocytes in blood, lung and BALF were differentiated and clinical parameters measured. Phage titers were quantified in plasma, BALF and lung. For Phage4Cure, a small number of phage candidates with broad host spectra against respiratory strains of *P. aeruginosa* was selected and transferred to the Fraunhofer ITEM (Institute for Toxicology & Experimental Medicine, Braunschweig) for pharmaceutical GMP production.
Results: We could expand our DSMZ phage collection of phages lytic for bacteria known to be pathogenic colonizers in the upper and lower airways including the gram-negatives *A. xylosoxidans*, *A. baumannii* and *P. aeruginosa*. These are in focus of the projects presented here. It could be demonstrated that our self-isolated *A. xylosoxidans* phages are morphologically and genetically diverse, highly lytic and attractive for therapeutic use. The *A. xylosoxidans* strains we used for testing the phages proved to be unexpectedly antibiotic-resistant. For investigating the lytic phage potential in the preclinical pilot study, the pathogenic *A. baumannii* strain RUH2037, global clone 1, was used with one single phage, Acibel004. Results were convincing: phages could be nebulized without much titer loss, no undesirable effects occurred 24h and 48h p. i., phage-treated animals had better clinical outcome, phages were detected in plasma, BALF and lung and no alterations in cell populations were detected. Data could be generated that will

contribute to the concept of the clinical application of pharmaceutically purified phages against *P. aeruginosa* in Phage4Cure.

Conclusion: While remarkable scientific progress is currently recorded in the field of phage biology and application, further research is deemed necessary by internationally renowned researchers to better understand phage action in vitro and in vivo. Chronical infection of the airways is a frequent medical complication that requires therapy options additionally or alternatively to antibiotics. Our experimental work so far could affirm that well-selected purified phages are capable to be applied as an alternative route to combat AMR bacteria.

Session II

New concept in Cystic Fibrosis care in 2019

Isabelle Sermet-Gaudelus

Introduction: Amazing progress has been made in the past decade in the discovery of cystic fibrosis transmembrane conductance regulator (CFTR) modulators and their rapid advancement to clinical trials. We now know that improving CFTR function at the molecular level is decisive in changing the course of the disease. This new paradigm heralds a new era in CF care.
Methods: Evolving knowledge of the molecular mechanisms responsible for defective CFTR has prompted new research focused on “repair” of each step of the CFTR defect, providing the proof of concept of a necessary combination therapy. It is however still unknown whether this “repair” of CFTR also corrects the multiple cellular consequences due to CFTR biogenesis defect.
This is the reason why mutation independent strategies are being developed. They are based on recent advances in gene editing technologies, such as the type II bacterial CRISPR/Cas9 (clustered regulatory interspaced short palindrome repeats/CRISPR associated). It may be possible, as a permanent cure, to insert bronchoalveolar stem cells that have been harvested and corrected with CRISPR/Cas9 into the stem cell niches in the lungs of CF patients. This strategy is yet in preclinical development, and must carefully address potential out-target effects. Similarly , tools are being developed , most at the preclinical step, to tackle specific molecular derangements, such as read-through molecules, splicing defect repairing tools, CFTR "amplifiers," or CFTR interactome modulators
Results: Within the next five years, the vast majority of patients with CF are expected to have a modulator approved for their genotype. Before considering prescription of such treatments in new-borns, and even in utero, with the dream that targeting the basic disease prevents organ damage, long-term efficacy and, above all, long-term tolerance have to be determined. This is clearly one of the most important challenges in the future. Indeed, these new “CFTR chaperone” molecules could also bind to other nearby, non specific, targets. Moreover, as these drugs possibly impact on intrinsic proteic properties and cellular pathways necessary for proper folding of many other proteins, they may likely have non-specific off-target effects which may be revealed after long periods of time. Fortunately, the CF community is in an ideal position for long term phase 4 safety monitoring because of the robust registries world-wide, which should be interoperable, permitting the vast majority of patients internationally to be followed closely for years. Indeed, with the increased speed and decreased cost of data processing, we are increasingly able to use a systems biology approach combining data analysis from registries and “omics” strategies to better understand mechanisms of action of the drugs, detect possible side or long term effect, and classify patients according to their specific omic background in a stratified medicine approach. It may thus be that in the future, initiation of treatment could be tailored to individual responses simply by testing in vitro the compound on patient derived primary culture. Although science progress at high speed, CF care must be patient centered. Digitization of health care, emphasis on outpatient care, and increase in home health care will be the cornerstones of daily care. Very importantly, shared decision-making approach must be developed to increase patient autonomy and participation in the medical decision-making process.
Conclusion: Such multidisciplinary advances, combining basic research and care delivery provide rationale for a personalized medicine strategy tailored for every CF patient in the very near future.

Session III

Comparison of efficacy of functional rescue of F508del CFTR by approved CFTR modulators

Constanze Vitzthum

Introduction: The F508del CFTR mutation is the most common CF-causing mutation. Up to 90 % of CF patients, carrying F508del at least on one allele and approximately 50% are homozygous for this mutation. Currently there are three approved CFTR modulators for the treatment of certain CFTR mutations available: 1) CFTR potentiator ivacaftor for gating and residual function CFTR mutations, 2) CFTR corrector lumacaftor in combination with ivacaftor for CF patients homozygous for F508del CFTR, and 3) CFTR corrector tezacaftor in combination with ivacaftor for CF patients with at least one copy of F508del CFTR.
However, the relative effectiveness of these CFTR modulators is not tested so far. The aim of this study therefore was to investigate the efficacy of the functional rescue of F508del CFTR in cystic fibrosis bronchial epithelial cells (CFBE) by the clinical relevant CFTR modulators ivacaftor, lumacaftor and tezacaftor (single and/or combination treatment).
Methods: To achieve this goal, CFTR-mediated currents were electrophysiologically assessed in Ussing chambers on either wildtype CFTR or F508del CFTR CFBE cells grown on permeable supports at liquid/liquid interface. 48h prior the Ussing chamber measurements vehicle (0.1% DMSO) and/or compounds (1 µM ivacaftor, 5 µM lumacaftor, 5 µM tezacaftor) were added to the culture media. In addition, F508del CFBE cells were incubation 48h at 27°C to induce low temperature rescue of F508del CFTR. In Ussing chamber experiments forskolin (1 µM) and IBMX (100 µM) were applied to induce cAMP activation of CFTR. To further activate CFTR ivacaftor (5 µM) was applied followed by the application of the CFTR channel blocker CFTRinh-172 (20 µM).
Results: Ongoing studies indicate that all three tested CFTR modulators activate WT and F508del CFTR in CFBE cells. Lumacaftor/ivacaftor more effective than tezacaftor/ivacaftor and lumacaftor alone followed by ivacaftor alone. The observed currents induced by CFTR modulators are sensitive to CFTRihn-172. The results of this ongoing study will be presented at the meeting.
Conclusion: We predict this study will provide important information on the relative effectiveness of functional rescue of F508del CFTR that may contribute to an optimized use of CFTR modulators in patients with CF.

Patient registry and implications for phase IV trials

Lutz Nährlich

Patient registries are committed to contribute to the real-world evidence of new medicines and scientific progress. In 2015 the European Medical Agency explore ways to expanding the use of patient registries to the benefit-risk evaluation of medicines. The European Cystic Fibrosis Society Patient Registry and national registries received a qualification opinion in 2017. Several European CF registries fulfilled the data quality requirements of the EMA and have contributed to pharmacovigilance studies over the last years. Two actual studies give an insight in the new challenges for patient registries: 1) In UK the randomized registry trial "CF-Start" assess the safety and efficacy of fluxocallin as a long-term prophylaxis agent for infants with CF in 63 centres. 2) In Australia BEAT-CF – a randomized, embedded, multi-arm adaptive platform trial for Pulmonary Exacerbations of Cystic Fibrosis is carried out. Patient registries offer a wide range of phase IV trials including PASS and randomized registry trials.

Choline supplementation to treat severe hepatosteatosis in a compound heterocygous CF patient

Wolfgang Bernhard

Introduction: In Cystic Fibrosis (CF) patients, exocrine pancreas insufficiency results in major fecal losses of choline. Choline is an essential nutrient and constitutive component of parenchyma, with tightly regulated tissue concentrations in the form of phosphatidylcholine (PC) & sphingomyelin (SPM). Hence, an adequate choline status is mandatory to organ function. Choline deficiency in CF is due to the high secretion rate of hepatic phosphatidylcholine (PC) via bile, and dysfunction of its enterohepatic cycle due to pancreatic phospholiase A2 (pPLaseA2) deficiency. Organs with high choline turnover, like the liver and lungs, are most affected by such deficiency, due to its role in tissue function & recovery. The background for this is the role of choline/PC for membrane formation, and -putatively- its role in apoptosis via ceramides, namely sphingomyelin synthesis from ceramides and PC.
In line with this, plasma choline and PC concentrations correlate with lung function and systemic inflammation parameters. Hence, we investigated in a pilot study the effect of choline supplementation (3x1g choline chloride) on choline pools, lung function and liver fat. Treatment resulted in normalized plasma choline concentration (10-15 vs. 6µM), improved $ppFEV_1$ (from 70(51-75)% to 78(60-84)% ($p<0.05$), and decreased liver fat from 1.6(0.4-8.8)% to 0.8(0.6-1.2)% ($p<0.01$). Based on these results, we supplemented a 23y old female CF patient (F508del/del exon 17a+17b) with severy hepatomegaly & steatosis & beginning fibrosis with choline.
Methods: Choline supplementation was achieved with 3g choline chloride per day in the form of 3x4mL of a 25% solution, dissolved in 250mL juice together with regular meals. Compliance was routinely controlled by telephone visits, and the first regular visit was after three months. Routine clinical assessment, laboratory parameters, and liver diagnostics by magnetic resonance spectroscopy was performed before and during treatment.
Results: Tolerance and compliance: The patient reported good tolerance of choline intake, with the exception that patient-friendly capsules would make her compliance significantly easier during working time. No adverse effects were reported during telephone or clinic visits. The patient reported relief from upper abdominal pain within two weeks after start of supplementation.

Liver: Initially, liver size was 2381mL, and fat 28.5%. After three months choline treatment, liver volume had decreased to 1921mL, fat to 8.8%, and liver structure appeared more homogenous.
Clinical chemistry: Plasma choline rose from 1.9 to 5.4µL (control: 10.2+/-0.4µM; other CF patients without supplementation: 5.9+/-2.0µM), and betaine from 6.5 to 23µM (control:25.6+/-9.1µM; other CF:23.1+/-10.5mM).
Trimethylamine oxide, an indicator of bacterial choline degradation, was high before (16.7µM) and after supplementation (17.7µM; controls: 2.3+/-0.7µM; other CF:5.6+/-5.3µM), indicating intestinal choline degradation prior to supplementation, but not as a result of supplementation.
HDL and LDL cholesterol increased from 24 & 59mg/dL, respectively, to 43 & 69mg/dL, respectively. GOT/AST decreased from 45 to 24 U/L, GPT/ALT from 93 to 47U/L, AP from 180 to 119U/L and gGT from 57 to 45U/L. During further choline treatment, AST, GPT, AP and gGT further decreased to 16, 28, 94 & 23U/L.
Conclusion: The data suggest that choline supplementation in the chosen dosage (2200mg/d), divided into three equal portions, dissolved in 250mL volume and taken together with a meal was well tolerated and effective in improving the choline status of this CF patient. Magnetic resonance spectroscopy confirmed efficacy on hepatosteatosis, improved liver texture and parenchymal integrity (decreased AST, ALT, AP, gGT). The initially very low plasma choline level (<2µM), the unassertive increase in plasma choline (5.4µM rather than 10-15µM as in other patients) & the high initial TMAO levels suggest that bacterial colonization of the intestine, resulting in choline degradation, may be an additional problem in this CF patient. While this case reports successful relief from severe hepatosteatosis by choline supplementation, further research on additional factors impacting on choline deficiency in CF is inevitable.

Keynote-Session

The CF Canada-Sick Kids Program in individual CF therapy: Towards personalized medicine in CF

Paul Eckford

Therapies targeting the major mutant in CF, F508del-CFTR, have been approved for use in patients (ORKAMBI and SYMDEKO), yet patient-to-patient variations in clinical response highlight the need for in-vitro functional and genetic tools that predict patient-specific clinical outcomes. It is likely that in the future, there will be several modulator therapies available on the market and there will be a need to understand, before treatment, which small molecule modulator will be most effective for each individual. The goal of the CF Canada-SickKids Program in Individual CF Therapy (CFIT Program) is to stimulate the development of new therapies for CF mutations where no effective therapy currently exists, and to develop methods to identify the best therapy for each individual, where more than one therapy is eventually marketed. Toward this goal, the CFIT Program is generating what we believe to be the most comprehensive internationally accessible data and biobank from a cohort of 100 CF individuals. This resource includes those individuals homozygous for F508del (in particular those starting modulator therapy, allowing us to sample pre- and post-drug and track the patient clinically), some heterozygous individuals and a large cohort of rare homozygous individuals, including those with splicing mutations (e.g. 621+1G->T), processing mutations (N1303K, G85E, M1101K), individuals with stop mutations (W1282X, G542X, etc.), and others where no modulator therapy currently exists. Recruitment is ongoing with samples collected from over 85 individuals to date, focussing primarily on those bearing homozygous mutations to avoid confounding results on the functional consequences of mutations and their response to modulators. For each individual we are banking high quality nasal cell cultures, generating well-characterized induced pluripotent stem cells (iPSCs), performing RNAseq on non-cultured primary nasal tissue, undertaking whole genome sequencing studies using newer long-read technologies that allow us to reconstruct phased genomes, and collecting clinical measures if the individual starts on modulator therapy. Each component of the CFIT Program is of high quality. For example our iPSCs are generated under best practice SOPs at the commercial CCRM facility, and DNA and RNA are extracted in a CLIA-certified clinical diagnostic laboratory. We are making these data and cell resources available to the CF research community and have shared cell samples and data with CF researchers in Canada, the US and internationally. Our thoroughly characterized iPSCs are being differentiated into a variety of CF-affected tissues (lung, bile duct, intestine, etc.). We are simultaneously improving our tissue models developed with these CF iPSCs, and developing higher throughput functional assays employing these tissues to study CFTR function, with the goal of defining the most robust models for future drug development. The scalability and reproducibility of these stem cell models will be evaluated as will the fidelity with which they recapitulate patient specific responses observed in primary tissues. While our goal is to stimulate the development of new therapies and to develop methods to identify the best therapy for each individual, longer term our iPSC resources should prove valuable as models to support the development of new methods to treat CF patients such as genetic correction and to study the challenges of returning genetically corrected cells to the body.

Session IV

Suitable clinical biomarkers for safety and long-term efficacy evaluation and current possibilities

Mirjam Stahl

Who wouldn't love an universally applicable biomarker that is suitable for every patient and answers all of our questions, especially those on safety and efficacy of a new treatment? Despite reality being a little more complicated than this dream, there are biomarkers available yet. This presentation will review the currently available biomarkers concerning their ability to detect long-term efficacy and/or safety in CF patients and will highlight open questions that need to be answered. Possible difficulties with current and also future biomarkers will be described to be addressed in upcoming studies minimizing the potential bias of these techniques.

Ivacaftor Therapy on Cystic Fibrosis Transmembrane Conductance Regulator Function is evaluated in a patient with 3849+10kbC>T mutation

Yin Yu

Introduction: The CFTR potentiator ivacaftor has been approved by the FDA for CF patients with gating and residual function CFTR mutations. It has been shown that intestinal current measurements (ICM) are sensitive to detect in vivo activation of CFTR in CF patients with G551D mutations after initiation of ivacaftor therapy. However, data on its effects in CF patients with the splicing mutation 3849+10kbC>T are pending. Therefore, we are evaluating the degree of functional rescue and clinical benefit of ivacaftor treatment in a patient carrying this mutation.
Methods: The patient is compound heterozygous for the CFTR mutations I507del and 3849+10kbC>T, resulting in CF with residual function anticipated with a milder progression in comparison to “classical” CF. However, there has been a significant worsening of CF lung disease. Due to severity and progression of the symptoms, we decided to treat the patient with ivacaftor since current experimental and clinical data indicate that the CFTR mutation 3849+10kbC>T is likely to respond to administration of a potentiator. To evaluate the effects of this therapy we monitored laboratory values, sputum, lung function, sweat test and clinical condition. Additionally, we investigated sensitivity of other biomarkers to detect in vivo activation of CFTR in patients with this mutation. Therefore, nasal potential difference measurement (nPD) and ICM were performed before and four months after start of therapy.
Results: After four months of therapy patient gained a total of 6,1 kg, resulting in an increase of body mass index of 2,3 kg/m2. Lung function was reevaluated, showing a normalization of lung clearance index after start of therapy. Side effects, e.g. elevation of liver enzymes, were not detected after four months of therapy. Sweat test was performed, showing a slight reduction of the mean chloride concentration from 51.5 mmol/l before ivacaftor therapy to 47 mmol/l after initiation of ivacaftor therapy. At baseline cAMP-dependent stimulation induced residual chloride secretory responses as well as inverse (positive) current responses reflecting potassium secretion, characteristic of residual CFTR chloride channel function in intestinal current measurements. Initiation of ivacaftor therapy resulted in a slight increase of cAMP-mediated and carbachol coactivated negative short-circuit current (Isc), reflecting augmentation of chloride secretion, compared with baseline in rectal tissue. However, no improvement was measured in nasal potential difference studies.

Conclusion: In addition to the improvement of the pulmonary function test by multiple breath washout, weight gain under CFTR-modulating therapy was also observed. In line with clinical improvement, the sweat test showed a reduction of the mean chloride concentration and a slight improvement of the CFTR function in the rectal mucosal biopsy after initiation of therapy. However, in nasal mucosa this effect was not detectable. Overall, we assume a response to CFTR-modulating therapy with ivacaftor with no signs of side effects. To our knowledge this is the first case to evaluate, whether nPD and ICM are sensitive biomarkers to detect in vivo activation of CFTR in patients with a 3849+10kbC>T mutation (without a G551D mutation) under ivacaftor treatment. We suggest that a larger number of patients with CF with splicing mutations producing low levels of normal CFTR transcripts should be evaluated.

Effects of Lumacaftor-Ivacaftor Therapy on Lung Disease Detected by Magnetic Resonance Imaging in F508del Homozygous Patients with Cystic Fibrosis

Simon Y. Graeber

Introduction: Recent studies suggest that lumacaftor-ivacaftor restores the activity of the cystic fibrosis transmembrane conductance regulator (CFTR) channel function to about 10 to 20% of normal CFTR activity and subsequently improves lung function in F508del homozygous patients with cystic fibrosis. However, the effect of lumacaftor-ivacaftor on the morphology of the lung remains unknown. The primary aim of this study was, therefore, to assess the short-term effects of lumacaftor-ivacaftor on lung morphology detected by MRI.
Methods: We performed lung MRI in 14 F508del homozygous patients older than 12 years at baseline and 8 to 16 weeks after initiation of therapy with lumacaftor-ivacaftor.
Results: At baseline, abnormal morphology was detected in all patients with a mean score of 14.7 (±7.4). MRI morphology score was significantly decreased by therapy with lumacaftor-ivacaftor to 13.4 (±7.4, p=0.025). Lung perfusion detected by MRI significantly improved from a MRI perfusion score of 6.6 (±2.6) at baseline to 5.4 (±3.5, p=0.038) after initiation of treatment with lumacaftor-ivacaftor. Further, the global MRI score was significantly improved from 21.3 (±9.6) at baseline to 18.8 (±10.5, p=0.012) by therapy with lumacaftor-ivacaftor.
Conclusion: Our results indicate that MRI detects improvement in lung morphology and perfusion in F508del homozygous patients treated with lumacaftor-ivacaftor. This study supports the use of MRI as a sensitive and radiation-free endpoint in clinical trials with CFTR modulators.

Effects of short-term Lumacaftor-Ivacaftor therapy on lung microbiome in Phe508del homozygous patients with cystic fibrosis

Sébastien Boutin

Introduction: The interplay between cystic fibrosis (CF) airways microbiota and the disease severity and CFTR malfunction were demonstrated by several studies. The discovery of CFTR corrector and potentiator was a breakthrough discovery but the influence of those therapies on the microbiome of the lung is still not fully explored. The aim of our study was to analyze the short-term effects of lumacaftor-ivacaftor on the lung microbiome in Phe508del homozygous patients.
Methods: 15 patients were included in this study sputum was use to sampled the lung microbiota at baseline and 8 to 16 weeks after initiation of therapy with the approved dose of lumacaftor 400 mg in combination with ivacaftor 250 mg every 12h. Samples were aliquoted (200 µL) and treated with PMA to avoids subsequent PCR amplification of extracellular DNA. DNA extractions were performed using the QIAamp Mini Kit. Microbiome was explored by amplifying the the V4 region of the 16S rRNA gene (515F and 806R) and PCR amplicons were sequenced in a Illumina Miseq sequencing system with 250 cycles. In parallel, the number of 16S copies was quantified by quantitative PCR (qPCR). Sequences were analyzed with the R package dada2. Raw sequences were filtered and trimmed for good quality and merge as contigs. Ribosomal sequence variants (RSV) were assigned to taxonomy using the Silva database (version 132). RSV assigned to eukaryotes, archaea and chloroplast were removed from the analysis.
Results: *Pseudomonas aeruginosa* was present in five patients at baseline and no change was seen through therapy with lumacaftor-ivacaftor. Other common genera found in the cohort were *Staphylococcus, Neisseria, Veillonella and Prevotella*, but no significant changes in single generas were observed under therapy. Nevertheless, treatment with lumacaftor-ivacaftor significantly increased the alpha-diversity of the microbiome of Phe508del homozygous patients. This effect was mostly due to a significant increase in the richness after lumacaftor-ivacaftor therapy. Dominance as the relative abundance of the most dominant RSV and the overall bacterial burden showed tendencies of reduction under therapy.
Conclusion: Those results indicates that the combination of lumacaftor and ivacaftor in Phe508del homozygous patients influence the microbiome of the lungs. This influencerelate in an increase of the α-diversity not due to the decrease of the most abundant RSV (dominance) but mostly to an improved colonization by the rare biosphere. This rare biosphere is mostly constituted of anaerobes like *Prevotella, Veillonella* and aerobic commensals like *Neisseria*. This would indicate that the CFTR restoration allow less stringent selection pressure on the migrants from the upper airways by a modification of the ecological niche. It was previously shown in several studies that a more diverse microbiome in the lower airways was correlated with a better lung function and milder disease. The absence of decrease in the pathogen load would indicate that CFTR modulation alone do not allow eradication of the infection however the increase in the diversity would indicate that an early CFTR modulation might prevent or delay the first infection by conserving a diverse microbiota in the lower airways offering potential competitors against the gram-negative pathogens.

Relationship between microbiome dysbiosis, inflammation neutrophil recruitment and lung function in CF patients

Dario L. Frey

Introduction: The alteration of the airway environment in cystic fibrosis (CF) patients is triggered by a mutation of the cystic fibrosis transmembrane conductance regulator (CFTR) gene. This favors the establishment of a chronic bacterial lung infection. Therefore, a better understanding of the link between the microbiome of the airways and the phenotype of the patient is crucial. In the last years most of the studies have focused on exacerbation or the lung function as a primary phenotype. However, an important aspect of the microbiome-host interplay is the inflammatory response to the infection. The aim of our study was to analyze the interplay between the CF patient airway microbiome, pro-inflammatory cytokines as well as neutrophil recruitment.

Methods: Spontaneous sputum samples of 77 clinically stable CF patients were analyzed by 16S amplicon sequencing targeting V4. Sequences were analyzed using the package dada2 to guarantee a good estimation of the error rate of each run, to trim reads for good quality and to remove chimeas. Ribosomal sequence variants (RSV) were assigned to taxonomy using the Silva database v132. In parallel cytokine measurements (IL1β; IL-8 and TNF-α) were performed by ELISA or Cytometric Bead Array (CBA) and FRET probe NEmo1 was used to measure the free neutrophil elastase (NE) activity.

Results: A diverse microbiome correlates with lower inflammation and better lung function. The α-diversity significantly correlates with levels of pro-inflammatory cytokines (IL-8 and TNF-α, $p<0.05$) and levels of free NE ($p<0.05$). The driving forces of the correlations are the evenness and dominance of the microbiome not the biomass. The α-diversity was strongly negative correlated to forced expiratory volume in one second percent predicted ($ppFEV_1$) and slightly positive with intrathoracic gas volume (ITGV) percent predicted. The more diverse the microbiome the better the lung function and the lower the level of air trapping. *Pseudomonas aeruginosa* were significantly linked to elevated levels of IL-8 ($p<0.05$) and tended to correlate positively to all inflammation parameters. Free NE levels negatively correlated to $ppFEV_1$. The patients of this study were divided into five clusters based on their microbiome structure. Three clusters show a high abundance of classical CF pathogens. The α-diversity was strongly decreased in clusters dominated by *P. aeruginosa*. Patients of these clusters had the lowest $ppFEV_1$ and the highest percentage of neutrophils in the lung.

Conclusion: The structure of the microbiota was linked to the inflammation and the lung function parameters. Dominance of *P. aeruginosa* within the microbiota triggers inflammation as well as decline in lung function the most. To our knowledge, our study is the first to link microbiota subtype to lung function, neutrophil count and activity as well as cytokine measurements in a cohort of CF patients.

Cystic fibrosis bronchial epithelial cells infected with *P. aeruginosa* biofilm at air-liquid interface for new anti-infectives tests

Justus C. Horstmann

Introduction: Chronic biofilm formation of *P. aeruginosa* is still a challenge in CF treatment, as classical anti-infectives fail in the course of CF patients´ life. We established a new co-culture model of CFBE41o- cells homozygous for ΔF508 mutation mimicking the cystic fibrosis airway together with a pre-formed *P. aeruginosa* biofilm. The aim is to create a predictive in vitro system for human CF lung infection in order to test novel anti-infectives.
Methods: Cells grown at the air-liquid interface on the Transwell® system are infected with a 72h pre-grown *P. aeruginosa* biofilm. Nebulization of Tobramycin or vehicle on the co-culture of biofilm and cells was done via Aerogen® Aeroneb® Lab Nebulizer after 1h of infection.
Results: Tobramycin (10µg dose) preserved barrier functions and cell viability after 4 and 24h treatment. CFU of *P. aeruginosa* biofilm was reduced by 3 and 4 logs, respectively. Infected cells treated with vehicle showed approx. 60% cytotoxicity after 4h and an increased release of IL-8; after 24h the cells did not survive the infection. To check the relation between bacterial killing and host cell survival, Tobramycin was nebulized at concentrations of 1µg and 100µg on infected cells. At 1µg dose, no reduction of CFU was observed whereas the host cells did not survive after 24 hours. The nebulization of 100µg efficiently killed almost all bacteria (10^1 CFU of surviving bacteria), whereas the host cells were still alive and showed preserved barrier function after 24h.
To assess if this infected co-culture could be suitable for successive antibiotic administration, 10µg Tobramycin was nebulized on infected cells previously treated for 24h with the same dose of antibiotic. Bacterial survival was checked after 24 hours as well as epithelial barrier integrity. Even with Tobramycin re-administration, the bacteria were not completely eliminated on the cells. Nevertheless, the host cells were alive, and showed a TEER of approximately 200 Ω x cm2.
Conclusion: In conclusion, the present model is a valuable system to test efficacy of antibiotics and other anti-infectives against *P. aeruginosa* as well as the host response to the treatment. Furthermore, this model allows to analyse host-response in a chronic state of infection.

Effect of hyperosmotic solutions on ion and water transport properties of airway epithelia

Hanna Schmidt

Introduction: The airway epithelium is covered by a thin apical liquid layer, the so-called airway surface liquid (ASL). Its regulation depends on complex mechanisms that involves transcellular and paracellular ion and water transport. In Cystic Fibrosis (CF) CFTR dysfunction causes reduction of ASL volume. Hyperosmotic inhalation therapy using sodium chloride solution (NaCl) and mannitol is considered to restore ASL volume via osmotic driven water transport. However, the underlying biophysical mechanisms of hyperosmotic inhalation therapy have been hardly investigated. This study aims to elucidate the effect of hyperosmotic solutions on transepithelial fluid transport and ASL regulation of the airway epithelium.
Methods: Transepithelial transport properties were studied in NCI-H441 (H441) cells and primary nasal epithelial cells (NEC). NEC were obtained from healthy subjects and CF patients and proliferated as conditionally reprogrammed stem cells. Both H441 and NEC were differentiated at air-liquid-interface conditions. Exposure to hypertonic solutions was performed by repetitive addition of hypertonic NaCl or mannitol solution to the apical surface of the epithelium for a four day period. ASL volume was measured via Deuterium oxide dilution method and ion transport was investigated in Ussing chamber experiments. mRNA transcript levels were determined by RT-PCR.
Results: Exposure of H441 epithelia to hypertonic solutions resulted in a 1.5-fold, 3.5-fold and 9-fold increase of ASL volume for 3% NaCl (1030 mOsm), 6% NaCl (2060 mOsm) and 20% mannitol solution (1100 mOsm), respectively. Obviously, increase in ASL volume was not directly proportional to the applied osmotic gradients. Similar effects were observed in NEC with a higher degree of ASL expansion. Volume increase was significantly higher when sodium resorption was blocked with amiloride. Exposure to isotonic NaCl or mannitol solution did not affect ASL volume. When hypertonic solutions were added to a single time point only, maximal ASL volume was observed after four to eight hours and ASL volume declined to baseline within 48 hours. Ussing chamber experiments revealed an 1.5-fold and 2-fold decrease of amiloride sensitive short circuit current for epithelia exposed to 6% NaCl and 20% mannitol solution, respectively. mRNA transcript levels encoding ENaC subunits were not affected. Furthermore, we observed a markedly reduced transepithelial electrical resistance (TEER) for epithelia exposed to 6% sodium chloride and 20% mannitol solution, indicating increased ion permeability of the paracellular diffusion barrier. Further experiments will determine effects on fluid transport capacity.
Conclusion: Our data show that apical exposure of airway epithelia to hypertonic solutions results in an increase of ASL volumes as expected. However, there was a non-linear relationship between ASL volume increase and applied osmotic gradients. Mannitol was more effective for ASL volume increase than NaCl solution. Probably, less ENaC mediated sodium resorption in Mannitol versus NaCl solution treated epithelia could explain the greater efficiency of mannitol treatment.

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Session V

Improving adherence by digital support – The transfer CF pilot trial

Anna-Maria Dittrich

Background: Treatment of cystic fibrosis demands a high treatment time investment with a particular focus on slowing the progression of lung function decline, which continues to be the most important contributor to morbidity and mortality. Adherence is challenging in CF due to the high treatment burden and the lack of immediate health consequences in case of non-adherence. Lung function decline in CF is particularly pronounced in the transition phase between 12 and 24 years of age. During transition, the improvement of self-management and self-responsibility on the one hand side, independence from the parents and the desire for normalcy on the other hand side are conflicting aspects for many adolescents with CF, which influence adherence to the time-consuming pulmonary therapy. Mobile health care applications (mHealth) could help to support self-management and independence and thereby reconcile seemingly conflicting goals to improve adherence, quality of life and ultimately CF life expectancy.
Objectives: A) To assess user behavior and satisfaction of adolescents and young adults with CF over a three-month observation period of using a mobile mHealth application focused on medication adherence and disease-investment. B) To identify areas of improvement for this mHealth application. C) To compare overall and disease-specific satisfaction, lung function and anthropometry of adolescents and young adults with CF before and after using the mHealth application.
Methods: Adolescents and young adults with CF (n = 27, 12-24 years, mean age 16 ± 3 years, 14 female, 11 male) used a free mHealth application for three months of which 25 provided questionnaire data for analysis at the end of the study. Data collection was carried out using questionnaires on usage characteristics and life satisfaction, and standardized assessment of lung function and anthropometry.
Results: The reminder function for medication was used by 15/21 (70%) of the participants at week four of the observation period. Use of the medication plan declined to 13/20 (65%) at eight weeks of the observation period. At the end of the study, only 4/23 (17%) of the participants wanted to continue using the application, suggesting low overall attachment to the application. Yet, 14/25 (56%) of participants saw the mobile application as a support for everyday life. Potential improvements targeting hedonistic qualities were identified to improve mHealth application adherence. Comparisons of satisfaction with different life aspects hinted at improvements for the sub-item “respiration” and more stable values for the sub-item “lack of handicap by CF”, suggesting that application use might stabilize certain CF-specific aspects of the weighted satisfaction with life. Lung function or anthropometry were not affected consistently, neither directly after the intervention, nor in two years following.
Conclusion: The majority of the patients did not want to continue using the application after the study period. Only a few CF-specific aspects of weighted life satisfaction were possibly stabilized by use of the mHealth application; clinical parameters FEV_1 and BMI were not affected. An adaptation of the functions to adolescent-specific needs could improve long-term use and (thus) positively affect the course of disease.

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Session VI

Functional and structural lung MRI to detect early abnormalities and change in cystic fibrosis

Jim Wild

MRI offers distinct advantages over CT for early disease detection in CF, being radiation free and capable of measuring both function and structure. Hyperpolarised gas ventilation MRI is highly sensitive to early lung disease and ^{1}H MRI sequences can be used to generate high-resolution images for detection of structural disease.
This invited talk will highlight the ability of different MRI sequences to detect CF-related lung abnormalities draw on work from the group in Sheffield with ^{3}He, ^{129}Xe and ^{1}H lung MRI studies in children and adults with cystic fibrosis.

Chairs

Andreas Hector
University Hospital Zürich
Zürich (Swiss)
andreas.hector@kispi.uzh.ch

Tümmler, Burkhard
Hannover Medical Scool
Hannover (Germany)
tuemmler.burkhard@mh-hannover.de

Stahl, Mirjam
University Hospital Heidelberg
Heidelberg (Germany)
mirjam.stahl@med.uni-heidelberg.de

Speakers/Moderators

Ballmann, Manfred
University Hospital Rostock
Rostock (Germany)
manfred.ballmann@rub.de

Bernhard, Wolfgang
University Hospital Tübingen
Tübingen (Germany)
wolfgang.bernhard@med.uni-tuebingen.de

Dittrich, Anna-Maria
Hannover Medical School
Hannover (Germany)
dittrich.anna-maria@mh-hannover.de

Frey, Dario L.
University Hospital Heidelberg
Heidelberg (Germany)
dariolucas.frey@med.uni-heidelberg.de

Horstmann, Julius
Saarland University
Homburg (Germany)
justus.horstmann@helmholtz-hips.de

Munder, Antje
Hannover Medical School
Hannover (Germany)
munder.antje@mh-hannover.de

Nash, Ed
Heartsland Hospital
Birmingham (United Kindom)
edward.nash@heartofengland.nhs.uk

Rosenecker, Josef
Alpenklinik Santa Maria
Bad Hindelang (Germany)
roseneckerj@santa-maria.de

Beekman, Jeffrey
University Medical Utrecht
Utrecht (The Netherlands)
jbeekman@umcutrecht.nl

Boutin, Sébastien
University Hospital Heidelberg
Heidelberg (Germany)
sebastien.boutin@med.uni-heidelberg.de

Eckford, Paul
Hospital for Sick Children
Toronto (Canada)
peckford@sickkids.ca

Gräber, Simon
Charité – University Hospital
Berlin (Germany)
simon.graeber@charite.de

Kahl, Barbara
Münster University Hospital
Münster (Germany)
kahl@uni-muenster.de

Nährlich, Lutz
University Hospital Gießen
Gießen (Germany)
lutz.naehrlich@paediat.med.uni-giessen.de

Rohde, Christine
Leibniz Institute
Braunschweig (Germany)
christine.rohde@dsmz.de

Schmidt, Hanna
University Hospital Ulm
Ulm (Gemany)
hanna.schmidt@uni-ulm.de

Schwarz, Carsten
Charitè – University Hospital
Berlin (Germany)
carsten.schwarz@charite.de

Sheppard, David N.
University of Bristol
Bristol (United Kingdom)
d.n.sheppard@bristol.ac.uk

Wild, Jim
University of Sheffield
Scheffield (United Kingdom)
j.m.wild@sheffield.ac.uk

Sermet-Gaudelus, Isabelle
Hospital Necker
Paris (France)
isabelle.sermet@nck.aphp.fr

Vitzthum, Constanze
Charité – University Hospital
Berlin (Gemany)
constanze.vitzthum@charite.de

Yu, Yin
University Hospital Heidelberg
Heidelberg (Germany)
yin.yu@med.uni-heidelberg.de

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