2018

European Cystic Fibrosis Society

15th ECFS Basic Science Conference

Conference Programme & Abstract Book

Loutraki, Greece

Chairpersons

Marcus Mall, Isabelle Callebaut and John F. Engelhardt

21 March – 24 March 2018

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CONFERENCE SPONSORSHIP & SUPPORT

The ECFS thanks the following for their support

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WELCOME FROM THE ECFS PRESIDENTS

Dear Friends and Colleagues,

It is a great pleasure to welcome you in Greece to the 15th European Cystic Fibrosis Conference entirely dedicated to Basic Science.

This year we are delighted to welcome Prof. Mall as the conference Chairperson who will be supported by Dr.Callebaut and Prof.Engelhardt as co-chairpersons.

Basic scientific discovery is critical to our understanding of CF, and already we can see new therapies coming through from the hard work of this endeavour.

The ECFS is committed to providing you with a platform to discuss your ideas with your colleagues during this important conference which will comprise a series of symposia with invited European and international guest speakers. It is a high priority for us to support the work of scientists in CF and we are proud that this conference is now established as a key annual event in the ECFS programme.

Your active participation will contribute to a productive exchange of information and we hope productive collaborations.

We extend a very warm welcome to an exciting conference.

Kris De Boeck
ECFS President

Isabelle Fajac
ECFS President Elect

WELCOME FROM THE CONFERENCE CHAIRPERSONS

Dear Friends and Colleagues,

We are happy to welcome you at the 15th ECFS Basic Science Conference taking place this year in Greece, the land of the Gods!
The cosmopolitan beach of Loutraki with its endless lacy coastline spreading out to the Corinth Canal is always full of life and surprises. The Canal was only completed in the late 19th century. It cuts through the narrow Isthmus of Corinth and separates the Peloponnese from the Greek mainland, arguably making the peninsula an island.

We think Loutraki will be the perfect place to engage in discussions in a relaxed atmosphere. At this conference, you can expect to hear about recent unpublished results, have vivid discussions of data and ideas in an informal and interactive environment

A key characteristic of the conference is the high attendance of young participants, both PhD students and post-docs, who contribute enormously to the success of this annual event with their enthusiasm. Here you will have excellent opportunities to discuss your data and interact in a great atmosphere with the best of European and international experts in this field.

This year's programme includes a number of symposia with international experts covering topical aspects of basic research in cystic fibrosis together with invited talks from submitted abstracts. There are also two keynote lectures, flash poster sessions, as well as a session from the ECFS Basic Science Working Group. On Saturday we will also have a Flash Paper session.

This combination has been extremely successful in the past and the conference offers a forum for informal brain-storming-type discussions.

We welcome all scientists, not just from the field of CF research, but also from a diverse range of related topics, to an exciting conference of high scientific quality.

Marcus Mall
Charite University Hospital Berlin
Germany

Isabelle Callebaut
Sorbonne University
France

John F. Engelhardt
University of Iowa
United States

2018 ECFS Conference

New Frontiers in Basic Science of Cystic Fibrosis

21-24 March 2018, Loutraki, Greece

Programme

Chairpersons:

Marcus Mall (Berlin, DE), Isabelle Callebaut (Paris, FR), John Engelhardt (Iowa City, US)

Wednesday, 21 March 2018 (Day 1)	
13:30-17:00	**Pre-Conference Seminar** **"Lung and gut microbiome: what has to be considered for CF"** **Chairs: Geneviève Héry-Arnaud (FR) / Stuart Elborn (UK)**
13:30-13:40	General introduction - Geneviève Héry-Arnaud (FR)
13:45-14:00	Area of technology in microbial diagnostics - Sebastien Boutin (DE)
14:05-14:20	Recent data on lung and gut microbiome - Michael Tunney (UK)
14:25-14:40	Pathophysiological factors of CF dysbiosis - Leah Cuthbertson (UK)
14:45-15:00	The effect of CFTR modulation on CF lung microbiota: What do we know and is it important? - Pradeep Singh (USA)
15:00-15:30	*Coffee break*
15:30-15:45	Modelling the microbiome - Julien Tap (FR)
15:50-16:40	Forum discussion in interaction with the audience
16:45-17.00	Wrap-up and next steps to move forward - Stuart Elborn (UK)
17:30-18:15	**Official Opening of the Meeting by the Conference Chairpersons** Followed by: New research opportunities for CF scientists: EU-OPENSCREEN and CORBEL Bahne Stechmann (DE)
18:15-19:15	**Opening Keynote Lecture** Pathogenesis and therapy of the muco-obstructive component of CF lung disease Richard Boucher (US)
19:15-20:00	**Welcome Reception**
20:00-21:30	*Dinner*

Thursday, 22 March 2018 (Day 2)	
07:30-08:45	*Breakfast*
08:45-10:30	**Symposium 1 – CFTR Expression, Folding, Trafficking and Activity**
	Chairs: Isabelle Callebaut (FR) – Christine Bear (CA)
08:45-09:10	CFTR structures, insights and horizons - Bob Ford (UK)
09:10-09:35	Interplay between NBD1 subdomain dynamics and CFTR coupled domain folding in cystic fibrosis - Gergely Lukacs (CA)
09:35-10:00	Folding and misfolding of CFTR variants - Ineke Braakman (NL)
10:00-10:10	Abstract 01 - What can we learn from structural models of CFTR? - Hanoch Senderowitz (IL)
10:10-10:20	Abstract 04 - Nanobodies as tools to stabilize different conformational states of CFTR - Maud Sigoillot (BE)
10:20-10:30	Abstract 09 - Escape from the ER: Mechanisms for Correction of CFTR Misfoldin - Douglas Cyr (US)
10:30-11:00	*Coffee break* & **Poster viewing**
11:00-12:45	**Symposium 2 – Cell Physiology and Ion Transport**
	Chairs: David Sheppard (UK) - Nicoletta Pedemonte (IT)
11:00-11:25	SPX-101 is a novel peptide therapeutic for the treatment of CF lung disease - Robert Tarran (US)
11:25-11:50	Role of CFTR and non-CFTR channels and transporters in airways surface liquid pH regulation - Michael Gray (UK)
11:50-12:15	TMEM16 proteins control mucus production and exocytosis - Karl Kunzelmann (DE)
12:15-12:25	Abstract 32 - Role of pendrin (SLC26A4) in secretion by primary bronchial epithelial cells - Dusik Kim (CA)
12:25-12:35	Abstract 33 - Early neonatal mortality of *Slc26a9* deficient mice is triggered by airway mucus obstruction - Pamela Millar-Büchner (DE)
12:35-12:45	Abstract 30 - ATP12A as an alternative therapeutic target in cystic fibrosis lung disease - Paolo Scudieri (IT)
12:45-14:30	*Lunch*
14:30-15:30	**Flash Poster Session (even numbers)**
	Chair: Nicoletta Pedemonte (IT)
15:30-16:00	*Coffee break &* **Poster viewing**
16:00-17:45	**Symposium 3 – Model systems**
	Chairs: John Engelhardt (US) - Margarida Amaral (PT)
16:00-16:25	Early airway disease in the CF pig model - David Stoltz (US)
16:25-16:50	Airway organoids - Jeffrey Beekman (NL)
16:50-17:15	Patient derived nasal cells, a new tool in translational research for Cystic Fibrosis? - Isabelle Sermet-Gaudelus (FR)
17:15-17:25	Abstract 45 - BMI-1 transduced basal vells as a renewable resource for air liquid interface cell culture models of cystic fibrosis - Stephen Hart (UK)
17:25-17:35	Abstract 51 - Development of a robust protocol for gene-editing human pluripotent stem cells - Sara Cuevas Ocaña (UK)

17:35-17:45	Abstract 49 - Nasal epithelial cells expansion and reprogramming with the anti-SMAD protocol to evaluate CFTR rescue for precision medicine - Ilaria Musante (IT)
17:45-18:00	*Break*
18:00-19:45	**ECFS Basic Science Working Group session:**
	Cystic Fibrosis: beyond the airways
	Chairs: Margarida Amaral (PT) – Jeffrey Beekman (NL)
	Intestine: modelling *in vitro* intestinal organs - Sylvia Boj (NL)
	Pancreas: CF-related diabetes - James Shaw (UK)
	Kidney: Disturbed kidney acid/base physiology in patients with CF - Jens Leipziger (DK)
19:45-21:30	*Dinner*
21:30-23:00	**Evening Poster Session:** Posters with Even numbers

Friday, 23 March 20187 (Day 3)	
07:30-08:45	*Breakfast*
08:45-10:30	**Symposium 4 – Mucus and Mucins**
	Chairs: David Stoltz (US) - Ric Boucher (US)
08:45-09:10	Unravelling the molecular mechanisms controlling respiratory tract mucus gel formation - David Thornton (UK)
09:10-09:35	Reporter Muc5b-GFP mouse to study Muc5b functions - Jean-Luc Desseyn (FR)
09:35-10:00	The biophysics of airway mucus in health and disease - Brian Button (US)
10:00-10:10	Abstract 56 - Intramolecular interactions of the airway mucin MUC5B and their role in packaging within secretory granules - Caroline Ridley (UK)
10:10-10:20	Abstract 59 - Identifying pathways regulating goblet cell metaplasia: phenotypic screening with bronchospheres - Henry Danahay (UK)
10:20-10:30	Abstract 71 - Lack of CFTR results in impaired innate immunity in small airways - Joseph Zabner (US)
10:30-11:00	*Coffee break* & **Poster viewing**
11:00-12:45	**Symposium 5 - Inflammation**
	Chairs: Bettina Schock (UK) - Rob Tarran (US)
11:00-11:25	Mechanisms of neutrophil activation in early cystic fibrosis airway disease - Rabindra M. Tirouvanziam (US)
11:25-11:50	Bacteria-driven peribronchial lymphoid neogenesis in cystic fibrosis and bronchiectasis - Pierre-Regis Burgel (FR)
11:50-12:15	Role of IL-1 receptor signaling in the pathogenesis and as potential therapeutic target in CF airway inflammation - Marcus Mall (DE)
12:15-12:25	Abstract 66 - L iminosugars: new anti-inflammatory drugs for CF lung disease?- Maria Cristina Dechecchi (IT)
12:25-12:35	Abstract 61 - ENaC has a pivotal role in modulating autoinflammation and glycolysis in cystic fibrosis - Thomas Scambler (UK)
12:35-12:45	Abstract 125 - Complex network involving miRNA and RNA-BP in CF - Alexandra Pommier (FR)

12:45-14:00	*Lunch*
14:00-18:30	**Free Afternoon**
18:45-19:45	**Flash Poster Session (odd numbers)**
	Chair: Alexandre Hinzpeter (FR)
19:30 -21:30	*Dinner*
21:30-23:00	**Evening Poster Session:** Posters with Odd numbers

Saturday, 24 March 2018 (Day 4)	
07:30-08:45	*Breakfast*
08:45-10:30	**Symposium 6 – Host-pathogen interactions**
	Chairs: Stuart Elborn (UK) - Pierre-Régis Burgel (FR)
08:45-09:10	Anaerobic bacteria in Cystic Fibrosis pulmonary infection - Michael Tunney (UK)
09:10-09:35	CF airways metagenome – Burkhard Tümmler (DE)
09:35-10:00	Invasive sampling of the lungs of children with CF suggests a revised model of CF infection pathogenesis - Pradeep Singh (US)
10:00-10:10	Abstract 77 - Intracellular persistence of *Pseudomonas aeruginosa* in airway epithelial cells and CF lungs - Dao Nguyen (CA)
10:10-10:20	Abstract 70 - Airway surface liquid acidification initiates host defense abnormalities in Cystic Fibrosis- Juliette Simonin (FR)
10:20-10:30	Abstract 58 - Using *Xenopus tropicalis* to develop a live *in vivo* model of mucociliary clearance - Eamon Dubaissi (UK)
10:30-11:00	*Coffee break* & **Poster viewing**
11:00-12:45	**Symposium 7 – Therapeutic Approaches**
	Chairs: Jane Davies (UK) - Pradeep Singh (US)
11:00-11:25	CFF therapeutics discovery beyond F508del - Martin Mense (US)
11:25-11:50	Recent advances in gene and base editing - Patrick Harrison (IE)
11:50-12:15	Development of a PI3Kγ-derived peptide as a standalone therapy to activate F508del-CFTR, limit lung inflammation and promote bronchorelaxation in Cystic Fibrosis - Emilio Hirsch (IT)
12:15-12:25	Abstract 90 - New correctors rescue F508del-CFTR activity at low nanomolar concentrations - Tiziano Bandiera (IT)
12:25-12:35	Abstract 87 - *In Vitro* Characterization of Clinical Stage Novel Corrector PTI-801 and Potentiator PTI-808 in Primary Airway Cell Models - John Preston Miller (US)
12:35-12:45	Abstract 86 - Restoration of CFTR function by Antisense Oligonucleotide splicing modulation - Batsheva Kerem (IL)
12:45-14:30	*Lunch*
14:30-16:15	**Symposium 8 – Translational CF Research**
	Chairs: Kris De Boeck (BE) - Isabelle Sermet-Gaudelus (FR)
14:30-14:55	Modeling patient specific responses to CFTR modulator therapies- Challenges and opportunities - Christine Bear (CA)
14:55-15:20	Translating molecular and cellular knowledge in treatments for Cystic Fibrosis - Margarida Amaral (PT)

15:20-15:45	Personalized medicine, Task Force update - Jane Davies (UK)
15:45-15:55	Abstract 116 - Drug repurposing for cystic fibrosis subjects with rare CFTR mutations - Eyleen de Poel (NL)
15:55-16:05	Abstract 119 - Effects of Lumacaftor-Ivacaftor Therapy on CFTR Function in Phe508del Homozygous Patients with Cystic Fibrosis - Simon Y. Graeber (DE)
16:05-16:15	Abstract 34 - SLC6A14 overexpression modifies ΔF508-CFTR function in human bronchial epithelial cells - Yu-Sheng Wu (CA)
16:15-16:45	*Coffee Break*
16:45-17:45	**Flash Paper Session**
	Chair: Mike Gray (UK)
16:45-17:00	IL-17A impairs host tolerance during airway chronic infection by *Pseudomonas aeruginosa* - Cristina Cigana (IT)
17:00-17:15	Quorum Sensing Down-Regulation Counteracts the Negative Impact of *Pseudomonas aeruginosa* on CFTR Channel Expression, Function and Rescue in Human Airway Epithelial Cells - Emmanuelle Brochiero (CA)
17:15-17:30	Analysis of long-range interactions in primary human cells identifies cooperative CFTR regulatory elements - Stéphanie Moisan (FR)
17:30-17:45	Anti-cystic fibrosis properties of the CB subunit of crotoxin: correction of ΔF508-CFTR chloride channel dysfunction - Grazyna Faure (FR)
17:45-18:00	*Break*
18:00-19:00	**Closing Keynote lecture**
	Pharmacological modulation of ion transport in CF: CFTR and beyond - Luis Galietta (IT)
20:30	*Dinner / Social Event*

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P2 Metadynamics simulations to study the transition between open and closed forms of the CFTR channel
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P3 Biophysical and structural studies of purified CFTR and the effects of the F508del and G551D mutations
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P67 Normalising inflammation in CF airways by DREAM inhibitors
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P68 Impact of F508del mutation on lipoxygenases expression and localisation in Cystic Fibrosis
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P69 Longitudinal analysis of the airways' microbiota in patient with Cystic Fibrosis
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P70 Airway surface liquid acidification initiates host defense abnormalities in Cystic Fibrosis
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P71 Lack of CFTR results in impaired innate immunity in small airways
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P72 Regulation of cathelicidin LL-37 and cyclooxygenase expression by *Pseudomonas aeruginosa* in human bronchial Cystic Fibrosis epithelial cells
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P73 *Pseudomonas aeruginosa* LasB subverts innate immunity in alveolar macrophages and lung epithelial cells
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P74 The Cif virulence factor perturbs airway defenses and exacerbates lung damage
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P75 Protease deficient *Pseudomonas aeruginosa* isolates are common in cystic fibrosis infections and cause exaggerated pulmonary inflammation
Shantelle Lafayette, Pierre Andre Casgrain, Chris Zealy, Daniel Houle, Danuta Radzioch, Simone Perinet, John Feltner, Ajai Dandekar, Alya Heirali, Michael Parkins, Simon Rousseau, Dao Nguyen

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P77 Intracellular persistence of *Pseudomonas aeruginosa* in airway epithelial cells and CF lungs
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P78 Inflammatory response of respiratory epithelial cells to *Aspergillus fumigatus*: a role of the Syk pathway in absence of Dectin-1 involvement
Viviane Balloy, Michel Chignard

P79 CFTR correction by lumacaftor+ivacaftor combo does not diminish rhinovirus RNA titer in primary cystic fibrosis bronchial epithelial cells
Nurlan Dauletbaev, Aparna Dintakurti, Larry C. Lands

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Murali Yanda, Hua Wang, William Guggino, Liudmila Cebotaru

P81 Characterization of edited CFTR message following efficient, targeted integration of CFTR superexon to correct CF-causing variants in and downstream of exon23
Kader Cavusoglu Doran, Karen Mention, David Sanz, Martina Scallan, Patrick Harrison

P82 Correction of multiple Cystic Fibrosis-causing variants by CFTR superexon homology-independent targeted integration (HITI)
David Jose Sanz, Karen Mention, Kader Doran-Cavusoglu, Jennifer Hollywood, Martina Scallan, Patrick Harrison

P83 Development of a nanoparticle formulation for delivery of a CRISPR/Cas9 gene therapy for Cystic Fibrosis
Amy Walker, Ileana Guerrini, Ahmad Aldossary

P84 Cultivation and characterisation of human airway epithelial cells with potential for CFTR editing for the development of cystic fibrosis therapies
Afroditi Avgerinou, Maximillian Woodall, Dani Lee, Demetra-Ellie Phylactopoulos, Ileana Guerrini, Chris C. Callaghan, Deborah Baines, Stephen Hart, Paola Bonfanti

P85 Curated database of candidate therapeutics for the activation of CFTR-mediated ion conductance (CandActCFTR)
Manuel Nietert, Sylvia Hafkemeyer, Frauke Stanke

P86 Restoration of CFTR function by antisense oligonucleotide splicing modulation
Yifat S. Oren, Ofra Avitzur, Efrat Ozeri-Galai, Michal Irony-Tur Sinai, Steve Wilton, Venkateshwar Mutyam, Yao Li, Steven M. Rowe, Aurélie Hatton, Anita Golec, Iwona Pranke, Isabelle Sermet-Gaudelus, Batsheva Kerem

P87 *In vitro* characterization of clinical stage novel corrector PTI-801 and potentiator PTI-808 in primary airway cell models
John Preston Miller, Soheil Aghamohammadzadeh, Adriana Villella, Daniel Parks, Tzyh-Chang Hwang, Ryan E. Tyler, Po-Shun Lee, Benito Munoz

P88 Advances in an amplifier-enhanced CFTR premature termination codon readthrough high throughput screen
Danijela Dukovski, Mandy Tam, Joshua Horan, Soheil Aghamohammadzadeh, Ryan Tyler, Po-Shun Lee, Benito Munoz, John P. Miller

P89 A novel CFTR potentiator with a distinct mutation-specific profile to existing CFTR potentiators identified using an amplifier-enhanced HTS
Soheil Aghamohammadzadeh, Daniel Parks, Adriana Villella, Tzyh-Chang Hwang, Ryan E. Tyler, John Preston Miller, Po-Shun Lee, Benito Munoz

P90 New correctors rescue F508del-CFTR activity at low nanomolar concentrations
Tiziano Bandiera, Federico Sorana, Francesco Berti, Alejandra Rodríguez-Gimeno, Nicoletta Brindani, Sine Mandrup Bertozzi, Giuliana Ottonello, Andrea Armirotti, Raffaele Spanò, Maria Summa, Rosalia Bertorelli, Ilaria Penna, Natasha Margaroli, Debora Russo, Emanuela Caci, Loretta Ferrera, Valeria Tomati, Elvira Sondo, Emanuela Pesce, Paolo Scudieri, Fabio Bertozzi, Nicoletta Pedemonte, Luis J.V. Galietta

P91 Structurally diverse Trimethylangelicin derivatives correct the primary defect in p.Phe508del-CFTR by stabilizing the first membrane-spanning domain
Onofrio Laselva, Giovanni Marzaro, Ilaria Lampronti, Serena Domenichini, Irene Muzzolon, Jasmine Tregnaghi, Anna Tamanini, Giuseppe Lippi, Roberto Gambari, Giulio Cabrini, Adriana Chilin, Christine E. Bear, Maria Cristina Dechecchi

P92 Role of ganglioside GM1 on CFTR stabilization at plasma membrane: a new challenge for the cystic fibrosis therapy
Giulia Mancini, Silvia Munari, Nicoletta Loberto, Maria Cristina Dechecchi, Giuseppe Lippi, Giulio Cabrini, Massimo Aureli, Anna Tamanini

P93 Exploiting a PI3Kγ Mimetic Peptide as a standalone drug to restore CFTR function, reduce inflammation and limit obstruction of the respiratory tract in cystic fibrosis
Alessandra Murabito, Kai Ren, Flora Pirozzi, Nancy L. Quinney, Deborah M. Cholon, Martina Gentzsch, Emilio Hirsch, Alessandra Ghigo

P94 RNF5 inhibitors as potential drugs for cystic fibrosis basic defect
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P95 Anion transport mechanisms through lipid bilayers by synthetic ionophores: towards a cystic fibrosis therapy
Claudia Cossu, Michele Fiore, Debora Baroni, Valeria Capurro, Emanuela Caci, Maria Garcia-Valcerde, Roberto Quesada, Oscar Moran

P96 Small molecule anionophores promote transmembrane anion permeation matching CFTR activity
Elsa Hernando, Valeria Capurro, Claudia Cossu, Michele Fiore, María García-Valverde, Vanessa Soto-Cerrato, Ricardo Pérez-Tomás, Oscar Moran, Olga Zegarra-Moran, Roberto Quesada

P97 Building *in vivo* potency and duration of action and into a novel inhaled ENaC blocker, ETD001
Henry Danahay, Clive McCarthy, William M. Abraham, Holly Charlton, Sarah Lilley, Roy Fox, Martin Gosling

P98 The ENaC regulatory peptide SPX-101 is resistant to proteolytic degradation in CF sputum
David Scott, Juliana Sesma, Bryant Wu, Timothy Stuhlmiller

P99 KvLQT1 K^+ channel activation improves the regeneration of human cystic fibrosis airway epithelium
Damien Adam, Laura Sognigbé, Anik Privé, Martin Desrosiers, Christelle Coraux, Emmanuelle Brochiero

P100 Development of novel ABCC4 inhibitors for potentiation of CFTR in human airway epithelial cells
Jenny P. Nguyen, Yechan Kim, Ryan D. Huff, Markus Heller, Jeremy A. Hirota

P101 Potassium channels as therapeutic targets in the repair of human cystic fibrosis airway epithelium in the presence of *Pseudomonas aeruginosa* exoproducts
Emmanuelle Brochiero, Claudia Bilodeau, Emilie Maillé, Mays Merjaneh, Simon Rousseau, Christelle Coraux, Damien Adam

P102 Red ginseng increases trans-epithelial chloride transport in CF and reduces *Pseudomonas aeruginosa* biofilm formation
Do-Yeon Cho

P103 Cationic antimicrobial peptides: an alternative and/or adjuvant to antibiotics active against multidrug-resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa*
Lhousseine Touqui

P104 Targeting IL-17-producing T cells attenuates the severity of *Pseudomonas aeruginosa* lung infection
Nicola Ivan Lorè, Barbara Sipione, Mededе Melessike, Jennifer A. Mertz, Jay Kolls, Alessandra Bragonzi, Cristina Cigana

P105 Treating acute and chronic *Pseudomonas aeruginosa* infection: what can we learn from mouse models?
Cristina Cigana, Serena Ranucci, Alice Rossi, Ida De Fino, Melessike Mededе, Alessandra Bragonzi

P106 NX-AS-401: a quorum sensing inhibitor that reduces virulence factor production, disrupts established biofilms and enhances the effect of tobramycin in multiple clinical isolates
Lucy Sykes, Danielle Williams, James Preece, Hajer Taleb, Almero Barnard, Harriet Oldham, Michael Graz, Graham Dixon

P107 Development of nanoparticles for silencing the beta-glucocerebrosidase GBA2 as a promising tool to reduce cystic fibrosis lung inflammation
Nicoletta N. Loberto, Paola P. Brocca, Domitilla D. Schiumarini, Giulia G. Mancini, Maria Cristina M.C. Dechecchi, Anna A. Tamanini, Giulio G. Cabrini, Giuseppe G. Lippi, Sandro S. Sonnino, Massimo M. Aureli

P108 Enhanced mucus diffusion with cationic nanoparticles by an alginate oligomer
Aristides Tagalakis, Dafni Gyftaki-Venieri, Mayuran Mathiyalakan, Francesca Drew, Philip Rye, Stephen Hart

P109 Stilbenoids from vine as treatment for Cystic Fibrosis ?
Elodie Serbine, Emilie Luczka, Michel Abely, Eric Courot, Jane Hubert, Jean-Hugues Renault, Myriam Polette, Michel Tarpin, Christelle Coraux

P110 Modulation of cellular membrane properties as a potential therapeutic strategy to heal pulmonary obstructive diseases
Linette Kadri, Ferru-Clément Romain, Amélie Bacle, Laurie-Anne Payet, Anne Cantereau, Reynald Hélye, Frédéric Becq, Christophe Jayle, Clarisse Vandebrouck, Thierry Ferreira

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P112 The CF Canada-sick kids program in individual CF therapy: A resource for the advancement of personalized medicine in CF
Paul Eckford, Jacqueline McCormack, Lise Munsie, Gengming He, Sanja Stanojevic, Sergio Pereira, Karen Ho, Julie Avolio, Claire Bartlett, Jin Ye Yang, Amy Wong, Leigh Wellhauser, Ling Jun Huan, Jiaxin Jiang, Hong Ouyang, Kai Du, Michelle Klingel, Lianna Kyriakopoulou, Tanja Gonska, Theo Moraes, Lisa Strug, Janet Rossant, Felix Ratjen, Christine Bear

P113 Exploring CF through variation landscapes
William Balch, Chao Wang, Salvatore Loguercio

P114 Dynamic changes of DNA methylation and pulmonary disease in Cystic Fibrosis
Milena Magalhaes, Jörg Tost, Fanny Pineau, Isabelle Rivals, Florence Busato, Nathan Alary, Laurent Mely, Sylvie Leroy, Marlène Murris, Davide Caimmi, Mireille Claustres, Raphaël Chiron, Albertina De Sario

P115 Personalized medicine for cystic fibrosis: functional characterization of rare CFTR mutations and their response to modulators in patient-derived materials and cellular models
Iris Silva, Sofia Ramalho, Nikhil T. Awatade, Raquel Centeio, Carlos M. Farinha, Margarida D. Amaral

P116 Drug repurposing for cystic fibrosis subjects with rare *CFTR* mutations
Eyleen de Poel, M.C. Hagemeijer, A.M. Vonk, H. Oppelaar, S. Heida-Michel, M.Geerdink, G. Berkers, P. van Mourik, C.K. van der Ent, J.M. Beekman

P117 A collection of intestinal epithelial organoids to support the development of drugs and diagnostics in Cystic Fibrosis by combining CFTR functional tests in personalized medicine
Sara Caldrer, Anna Baruzzi, Silvia Vercellone, Angela Sandri, Valeria Esposito, Federica Quiri, Alessia Farinazzo, Luca Rodella, Angelo Cerofolini, Francesco Lombardo, Filippo Catalano, Luca Frulloni, Laura Bernardoni, Baroukh Assael, Hugo deJonge, Claudio Sorio, Paola Melotti

P118 Limited rescue of W1282X-CFTR function by CFTR-restoring compounds in organoids
Marne Hagemeijer, Peter van Mourik, Eyleen de Poel, Sylvia Suen, Annelotte Vonk, Kors van der Ent, Jeffrey Beekman

P119 Effects of lumacaftor-ivacaftor therapy on CFTR function in Phe508del homozygous patients with Cystic Fibrosis
Simon Y. Graeber, Christian Dopfer, Lutz Naehrlich, Lena Gyulumyan, Heike Scheuermann, Stephanie Hirtz, Sabine Wege, Heimo Mairbäurl, Marie Dorda, Rebecca Hyde, Azadeh Bagheri-Hanson, Claudia Rueckes-Nilges, Sebastian Fischer, Marcus A. Mall, Burkhard Tümmler

P120 Ivacaftor potentiation of CFTR in healthy human adults: single gland sweat rate analysis
Jeffrey Wine, Jeeyeon Kim, Miesha Farahmand, Colleen Dunn, Milla Carlos, Rina Imari Horii, Ewart A. C. Thomas, Richard B. Moss

P121 CFTR protein as a predictive biomarker
Peter Bialek, Calvin Cotton, Maneet Singh, Melissa French, Po-Shun Lee

P122 Characterization of novel urinary exosomal biomarkers for monitoring the effects of CF pathophysiology in patients
Sébastien Gauthier, Vincent Jung, Rosa Coucke, Chiara Guerrera, Aleksander Edelman, Miriam Bujny, Isabelle Sermet-Gaudelus

P123 Follow-up of CF patients using DNA methylation markers and sputum samples
Fanny Pineau, Davide Caimmi, Isabelle Rivals, Isabelle Vachier, Jörg Tost, Milena Magalhães, Mireille Claustres, Raphaël Chiron, Albertina De Sario

P124 LncRNAs: emerging players in *CFTR* gene regulation
Jessica Varilh, Alexandra Pommier, Karine Deletang, Michel Koenig, Mireille Claustres, Magali Taulan-Cadars

P125 Complex network involving miRNA and RNA-BP in CF
Alexandra Pommier, Jessica Varilh, Michel Koenig, Mireille Claustres, Magali Taulan-Cadars

P126 Identification of SLC26A9 chloride channel activators by high-throughput screening
Anita Balazs, Aliaksandr Halavatyi, Johanna J. Salomon, Rainer Pepperkok, Marcus A. Mall

P127 Characterization of airway and bone marrow derived macrophages in a mouse model of cystic fibrosis
Kerstin Brinkert, Theresa Buchegger, Elena Lopez Rodriguez, Mania Ackermann, Burkhard Tümmler, Antje Munder

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ECFS Young Fellows Travel Award

Afroditi Avgerinou (UK)
Sara Cuevas Ocaña (UK)
Ahmad Elbahnsi (FR)
Marjolein Ensinck (BE)
Tamara Madacsy (HU)
Pamela Millar-Büchner (DE)
Juliette Simonin (FR)
Yu Sheng Wu (CA)

Student Helper Award

Onofrio Laselva (CA)
Iris Silva (PT)
Emese Tóth (HU)
Amy Walker (UK)

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Giulia Mancini (IT)
Alessandra Murabito (IT)
Ilaria Musante (IT)
Emanuela Pesce (IT)
Alexandra Pommier (FR)
Paolo Scudieri (IT)

Vaincre La Mucoviscidose Travel Award

Nesrine Baatallah
Mégane Collobert
Chloe Grebert
Réginald Philippe
Jessica Varilh

Mukoviszidose eV Travel Award

Giovana Bavia Bampi
Annika Schmidt

21 March — 18:15–19:15

Opening Keynote Lecture

Pathogenesis and therapy of the muco-obstructive component of CF lung disease

Richard C. Boucher

University of North Carolina at Chapel Hill, US

The term "muco-obstructive lung disease" describes well a syndrome that includes chronic bronchitis/COPD, primary ciliary dyskinesia (PCD), and non-CF bronchiectasis. The pathophysiologic sequence of these diseases appears to involve airway epithelial dysfunctions that increase mucus concentration and produce adhesive mucus/mucus plaque formation on airway surfaces. The earliest disease manifestations occur in the small airways with subsequent development of bronchiectasis. A central question for CF pathophysiology is how well it fits into this disease scenario and what therapies may be effective in muco-obstructive diseases.

CF will be systematically compared to the muco-obstructive paradigm. First, evidence will be reviewed with respect to a small airway vs. large airway initiation of CF disease and for disease progression. Second, the etiology of small airway mucus plaque and plug formation will be evaluated, *i.e.*, are plugs formed in small airways a result of local epithelial abnormalities or are they formed via retrograde movement of mucus from more proximal/gland-rich airways? Third, an analysis of the etiology of the mucus abnormalities that produce plug formation will be discussed. Hypotheses that describe abnormalities of mucus viscoelastic and osmotic properties will be discussed in the context of abnormal mucus pH vs. abnormal mucus concentration. Particular emphasis will be placed on the role of mucus hyperconcentration in producing abnormal CF mucus properties, including raised viscoelastic, osmotic, cohesive, and adhesive properties. Fourth, attempts will be made to link abnormalities in transepithelial ion and water transport to the formation of abnormally concentrated mucus. The concept CF airways epithelial transport/airway surface hydrating functions as "vulnerable" to outside insults will be developed. Emphasis will be placed on coordination of ion/water transport and mucin secretion rates mediated by airway epithelial regulatory pathways. A focus will be on the extracellular ATP/adenosine regulation of the balance of sodium vs. chloride secretion in health and in the context of viral infections and gastric aspiration. Finally, the coupling of inflammation-induced mucin hypersecretion and coordination of mucus dehydration in the context of abnormal autocrine/paracrine signaling from CF goblet cells to ciliated epithelia will be addressed.

Evidence from clinical studies of CF adults, including sputum percent solids measurements of hydration, mucin concentration, osmotic pressures, and *in vivo* mucus clearance and airway surface liquid absorption data, will be reviewed. To complement data from adult CF studies, data from studies of mucus, metabolomics, and microbiome from an observational study of neonatal/preschool children with CF cohorts, *i.e.*, the AREST CF cohort, will be presented.

Finally, the role of therapies for the muco-obstructive component of CF will be reviewed. The role of hydrating agents of a broad number of classes will be reviewed with the goal of restoring mucus to normal concentrations and, hence, biophysical parameters commensurate with restoration of mucus flow/clearance. In addition, the concept of normal mucus as a "reversible" gel vs. CF mucus exhibiting the properties of a "permanent" gel will be discussed. In this context, the need potentially for novel mucolytic agents to complement the effectiveness of hydrating agents will be explored.

22 March — 08:45–10:30

S01: CFTR Expression, Folding, Trafficking and Activity

S1.1 CFTR structures, insights and horizons

Xin Meng, Jack Clews, Ohoud Jefri, Eleanor Martin, Robert C. Ford

School of Biological Sciences, University of Manchester, Oxford Rd, Manchester M13 9PL, UK

CFTR is a chloride channel that has evolved from the ATP-binding cassette transporter family. After many years of effort aimed at obtaining a structure for the protein, there are now three cryo-EM derived structures at better than 4Å resolution, two in the dephosphorylated, quiescent state in the absence of nucleotide, one in the phosphorylated, activated state with nucleotide bound. Most of the structure has been revealed, although about 200 amino acid residues still remain unresolved. Novel aspects of the structures versus other ABC transporters will be reviewed and insights about the evolution of CFTR as an ion channel will be discussed. We will also compare the high resolution structures with a lower resolution structure for CFTR in the phosphorylated, activated state but with no nucleotide. Other biochemical and biophysical measurements on the purified CFTR protein will be presented and then interpreted using the available structural data for the channel. The prospects for using the high resolution structural data for informing CF therapy will be discussed.

S1.2 Interplay between NBD1 subdomain dynamics and CFTR coupled domain folding in cystic fibrosis

Naoto Soya[1], Ariel Roldan[1], Haijin Xu[1], Ryosuke Fukuda[1], Tamas Hegedus[2], Gergely L. Lukacs[1]

[1]Department of Physiology, McGill University, Montréal, Quebec H3G 1Y6, Canada and , [2]Molecular Biophysics Research Group of the Hungarian Academy of Sciences, Department of Biophysics and Radiation Biology, Semmelweis University, Budapest, Hungary

Cystic fibrosis (CF), the most common autosomal recessive diseases in Caucasians, is caused by the functional expression defect of the cystic fibrosis transmembrane conductance regulator (CFTR) anion channel. The most prevalent mutation, deletion of F508 residue (DF508), similar to other point mutations in the nucleotide binding domain 1 (NBD1), leads to global misfolding and ER degradation of CFTR. CFTR contains four structured domains (NBD1-2 and membrane spanning domains 1-2, MSD1-2) and the intrinsically unstructured regulatory (R) domain. The F508 residue is critical for the folding and stability of the NBD1 and the coupled domain folding by interfacing a hydrophobic pocket formed by the NBD1-cytosolic loop 4 (CL4). Despite extensive efforts, we still lack full understanding of CFTR global unfolding provoked by numerous CF-causing point mutations in the four major domains and the reversal of this phenomenon by combination of pharmacological chaperones that can rescue both the NBD1 and its interface defect. To uncover the spatiotemporal unfolding dynamics of NBD1 variants lacking second-site suppressor mutations and its link to the conformational defect of CFTR, we used hydrogen deuterium exchange and mass spectrometry (HDX-MS) with peptic digests of NBD1 and limited proteolysis of CFTR, respectively. HDX-MS of isolated native-like ΔF508- and WT-NBD1 shows an EX1-type HDX kinetics at multiple peptides of ΔF508-NBD1, indicating that the localized unfolding of the mutant restricted to the vicinity of the mutation vicinity at 25°C, propagates to all NBD1 subdomains at 37°C. The afflicted peptides unfold at ~10-15-fold faster kinetics than their WT counterparts. The unfolding starts at the a-subdomain, and spreads to the F1-like ATP-binding and b-subdomain, compromising the stability of multiple domain-domain interfaces of the ΔF508-NBD1 and other mutations. Based on the unfolding HDX kinetics, the half-life of newly translated ΔF508-NBD1 and WT-NBD1 is predicted to be ~50 to ~750 sec, respectively, in isolation at 37°C. This implies that the ΔF508-NBD1 is unable to be engaged in physiological domain-domain interactions, a prerequisite of CFTR cooperative domain folding. The short half-life of WT-NBD1 relative to the slow posttranslational domain folding of WT-CFTR with a half-time of ~10 min folding (NSMB 2005:12, 17-25) likely contributes to the limited folding efficiency of the CFTR channel. While second second-site suppressor mutations confined to multiple NBD1 subdomains are able to restore the global thermal stability of ΔF508-NBD1 to WT, they were short on correcting CFTR coupled domain folding, measured by HDX-MS, melting, and limited proteolysis, respectively. The latter inference is supported by the observation that sole disruption of energetic coupling between NBD1 and MSD2 folding was sufficient to compromise the global folding of CFTR without altering NBD1 the thermal stability. These observations underscore the role of coupled domain folding in the channel posttranslational biogenesis and help to outline a network of residue interactions that render NBD1 subdomains sensitive to kinetic and energetic stabilization, a prerequisite for CFTR coupled domain ensemble, which may be exploited to improve the efficacy of pharmacological chaperones in CF.

S1.3 Folding and misfolding of CFTR variants

Marjolein Mijnders, Bertrand Kleizen, Marcel van Willigen, Daniel Fonseca, Peter van der Sluijs, Ineke Braakman

Cellular Protein Chemistry, Faculty of Science, Utrecht University, The Netherlands

ABC transporters form a very large, clinically relevant family of proteins that facilitate transport across membranes. Mutations in ABC transporter CFTR cause the lethal genetic disease cystic fibrosis. Most disease mutations lead to misfolding of the protein and therefore cause a defect in the protein's transport and/or its functioning as chloride channel. We analyze these mutations and correlate biochemical, cell biological, functional, and clinical parameters. This has increased understanding of the domain folding and assembly of wild-type CFTR and the requirements for its export from the ER and its functionality.

P1

S1.4 What can we learn from structural models of CFTR?

Hanoch Senderowitz, Luba Simchaev, Michael Zhenin, Netaly Khazanov

Bar-Ilan University, Chemistry, Ramat-Gan, Israel

Until recently, the lack of high resolution structures of different conformational states of CFTR has largely impeded atomic-scale understanding of the structural, energetic and dynamics effects of CF-causing mutations as well as rational drug design of CFTR modulators. This situation has dramatically changed with the publication of the coordinates of Zebrafish and human CFTR, in a dephosphorylated, ATP-less, inward-facing closed conformation and those of Zebrafish CFTR in a phosphorylated, ATP-bound, outward facing, near-open conformation. These structures are important for several reasons. First they demonstrate that CFTR is amenable to structural studies, using cryo Electron Microscopy (cryo-EM). This finding has already initiated multiple efforts towards resolving additional conformations of the protein. Second, these structures highlight new structural features of CFTR which may prove relevant for drug discovery. At the same time, these structures largely validated previously developed homology models of CFTR. This may suggest that available models that correspond to other conformational states of the protein are likely to be correct. Third, the structures serve as a tool to validate and interpret decades of efforts focusing on biochemical and functional studies. Finally, the structures could be used to initiate structure-based drug design efforts and perhaps help in developing mutation-specific therapies.

Despite this impressive progress, multiple challenges still remain. (1) The available structures only provide a static snapshot of the highly flexible and allosteric CFTR. (2) It is still not known which of the multiple CFTR conformations is sensitive to drug binding. (3) It is still not known where on CFTR, drugs are likely to bind. All these questions could be at least partially answered by solving additional structures of CFTR either in the apo state or in the presence of known modulators. However, until multiple CFTR-ligand complexes become available, answers and / or test-able hypotheses could be provided by computational techniques.

With this in mind, we present in this work a series of molecular dynamics (MD) simulations initiated from the EM structures of CFTR for both the WT protein and some of its mutants. We demonstrate that an analysis of the resulting trajectories could provide information on the stability of the EM structures as well as the effect of mutations. We also demonstrate how MD trajectories could be used for suggesting potential binding sites for CFTR modulators, and how such modulators could be docked into these sites. We propose that the hypotheses put forth by the computational analysis could be experimentally tested, e.g., by monitoring the effect of strategically designed mutations on ligand binding and by testing the compliance of the suggested sites with Structure Activity Relationship (SAR) data.

P4

S1.5 Nanobodies as tools to stabilize different conformational states of CFTR

Maud Sigoillot[1], Magdalena Grodecka[1], Marie Overtus[1], Daniel Scholl[1], Abel Garcia-Pino[2], Lihua He[3], Els Pardon[4], Toon Laeremans[4], Jan Steyaert[4], John Riordan[3], Cedric Govaerts[1]

[1]ULB, SFMB, Brussels, Belgium, [2]ULB, Faculté des Sciences, Charleroi, Belgium, [3]UNC-Chapel Hill, Department of Biochemistry and Biophysics and Cystic Fibrosis Center, Chapel Hill, United States, [4]VIB-VUB, Structural Biology Research Center, Brussels, Belgium

In most cases, Cystic Fibrosis is due to the deletion of the residue phenylalanine 508 (F508) in the first nucleotide binding domain (NBD1) of CFTR that prevents folding of the whole protein leading to its clearance by the quality control system and defects in channel gating. It is well established that F508 mutation affects NBD1 thermodynamic stability and possibly the interface between the NBD and the trans-membrane (TM) domain. Thus, chaperones that target directly NBD1 are the most likely to recover protein stability, a first step towards recovering transport activity. To understand the mechanistic basis of CFTR stabilization, we developed nanobodies as tools to lock different conformations of CFTR. We identified different families of nanobodies that bind to distinct epitopes of NBD1 of CFTR and stabilize the protein in different conformational states. Using X-ray crystallography, we solved the structures of the complexes and identified the epitopes at the atomic level. Our approach leads to the identification of high affinity CFTR-specific stabilizers, which opens new avenues to decipher CFTR folding mechanism and develop CFTR correctors. In addition, our high-resolution structural data surprisingly demonstrates that CFTR must be able to adopt conformations that depart significantly from the published cryo-EM structures, further establishing that CFTR is a highly dynamic protein, even under normal physiological regime.

P9

S1.6 Escape from the ER: Mechanisms for Correction of CFTR Misfolding

Hong Yu Ren, Scott Houck, Andrew Kennedy, Radu Mitran, Lihua He, Douglas Cyr

UNC-Chapel Hill, Cell Biology, Chapel Hill, United States

Cystic Fibrosis (CF) is a fatal disorder associated with defective hydration of lung airways due to misfolding and premature degradation of the mutant Cl- channel F508del-CFTR. CFTR contains two membrane-spanning domains (MSD), two nucleotide-binding domains (NBD) and a regulatory domain. The formation of an active CFTR channel therefore requires: 1) proper subdomain folding, and 2) efficient subdomain assembly. F508del, which occurs in NBD1, reduces the thermodynamic stability of NBD1 and also hinders early and late biogenic steps required for CFTR assembly. Folding of F508del-CFTR therefore arrests at an intermediate state. Kinetically trapped F508del-CFTR folding intermediates are recognized by cytosolic Hsp70 and targeted for ERAD via the RMA1 and CHIP E3 ubiquitin ligase complexes. A subset of CFTR II mutants misfold and accumulate in a conformation that is recognized by Hsp70, but is difficult to degrade by ERAD. ERAD-resistant forms of misfolded CFTR enter complexes that contain ERAD machinery, but become stuck, so they must be cleared from the ER via a recently discovered ERQC-Autophagy pathway. Class I and Class II folding modulators act synergistically to suppress folding defects in CFTR and thereby accelerate the rate of CFTR folding, which enables F508del-CFTR biogenic intermediates to escape detection by ERQC machinery and traffic to the plasma membrane.

How misfolded CFTR is recognized by ERQC machinery is not clear. Likewise, the mechanisms by which Class I and II folding modulators alter the conformation of CFTR to promote its escape from the ER are largely unknown. We are addressing these questions to define the rate limiting steps in the folding of CFTR that must be overcome to increase the cell surface expression of F508del-CFTR and rare CFTR II mutants. Approaches taken to address these questions include cell based folding assays, structural modeling, and molecular dynamic simulations of CFTR assembly. Class I modulators were found to stabilize an N-terminal domain of CFTR that contains MSD1. Class I modulator action is dependent upon a network of amino-acids located at the interface between cytosolic surfaces ICL1 and NBD1. Class I modulators appear to stabilize the ICL1:NBD1 interface, which in turn helps stabilize F508del-NBD1. Class II folding modulators have little impact on the biochemical stability of MSD1, and instead promote the assembly of full-length CFTR into a conformation that is no longer recognized by the RMA1 E3 ligase. RMA1 interacts with DNAJB12 and Hsp70 to detect folding defects that appear to occur when F508del prevents the proper association of MSD1/NBD1 with MDS2. Models will be presented to describe how Class I and Class II modulators synergize to promote the folding/assembly of F508del-CFTR to a conformation can pass ERQC and traffic to the cell surface. This work was supported by grants from the NIH and North American CF Foundation.

22 March — 11:00–12:45

S02: Cell Physiology and Ion Transport

S2.1 SPX-101 is a novel peptide therapeutic for the treatment of CF lung disease

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In cystic fibrosis (CF) lungs, epithelial sodium channel (ENaC) hyperactivity causes a reduction in airway surface liquid volume, leading to decreased mucocilliary clearance, chronic bacterial infection and lung damage. Inhibition of ENaC is an attractive therapeutic option. However, ENaC antagonists have failed clinically due to off-target effects in the kidney and/or poor duration of action. The short palate, lung, and nasal epithelial clone 1 (SPLUNC1) is an abundant, 256 amino acid protein that is secreted into the airway lumen. SPLUNC1 negatively regulates the epithelial sodium channel (ENaC) through its N-terminus to limit ASL absorption (1). SPLUNC1 is an allosteric regulator of ENaC that binds extracellularly to ENaC and induces intracellular NEDD4.2-dependent ubiquitination and internalization of ENaC (2). We have previously demonstrated that pH-sensitive salt bridges on SPLUNC1 modulate the ability of SPLUNC'1's N-terminus to bind to ENaC, leading to ASL dehydration in CF airway epithelia. Taken together, these data suggest that SPLUNC1 replacement would serve to ameliorate CF ASL dehydration (3). SPX-101 is a novel, peptide mimetic of SPLUNC1 that induces ENaC internalization, attenuates transepithelial sodium absorption and increases ASL hydration in CF airway cultures (4). SPX-101 was also found to increase survival rates in β-ENaC-overexpressed transgenic mice that spontaneously developed lung disease and restored mucus transport in an ovine model where CFTR was pharmacologically inhibited (4). Preclinincal studies indicate that SPX-101 is not degraded by neutrophil elastase and, unlike SPLUNC1, is stable when added to CF sputum, which is highly proteolytic. We have previously demonstrated that SPLUNC1-binding to ENaC does not require CFTR (1). Similarly, recent data demonstrate that SPX-101 is mutation agnostic and can inhibit ENaC independently of the class of CFTR mutation. A common side-effect of inhaled small molecular ENaC-antagonists is that they inhibit ENaC in the kidney leading to potassium-sparing natriuresis and hyperkalemia. However, despite being stable in the CF lung environment, SPX-101 has very low and transient bioavailability and does not cause natriuresis or hyperkalemia in rodents and also did not cause dose limiting adverse effects in a Phase I study in healthy human subjects. Based on these data, clinical trials in CF patients are ongoing. In conclusion, inhaled SPX-101 appears to have an excellent safety profile and is a promising therapeutic that can restore ASL hydration and mucus clearance in a CFTR-mutation agnostic fashion.

Funded by the Emily's Entourage, the North Carolina Biotechnology Center, NIH/HNLBI and Spyryx Biosciences

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S2.2 Role of CFTR and non-CFTR channels and transporters in airways surface liquid pH regulation

Vinciane Saint-Criq, Michael Gray

Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne, UK.

Cystic fibrosis (CF) is caused by mutations in the gene encoding the CF transmembrane conductance regulator (CFTR) protein, an apical membrane Cl^-/HCO_3^- channel that controls the amount and composition of epithelial secretions. Absence of CFTR impairs transepithelial salt and fluid flow, predisposing tissues to disease. Much research has focused on the Cl^- permeability defect of CFTR and its correction in CF. However, this channel also conducts HCO_3^-, and importantly, regulates several plasma membrane proteins involved in H^+/HCO_3^- transport. There is now strong evidence that the lack of HCO_3^- secretion in CF airways leads to reduced airways surface fluid (ASL) pH, which in turn impairs bacterial killing, enhances sodium-absorption through the Epithelial Sodium Channel (ENaC), and negatively impacts mucus rheology. Other organs such as the pancreas, gut and reproductive tract are similarly affected by acid pH. *Therefore*, *normalization of the pH of CF secretions has the potential to yield major clinical benefit.* In order to identify new therapeutic targets for CF airway disease, we need to understand the contribution of both CFTR and non-CFTR transporters and ion channels to ASL pH regulation, in both non-CF and CF contexts. Using a combination of intracellular and extracellular (ASL) pH measurements, combined with transepithelial ion transport and mucus transport studies, from fully-differentiated primary human airway non-CF and CF cultures, our work has so far confirmed important roles for both CFTR and the H^+/K^+-ATPase (ATP12A) proton pump, but it has also identified several additional ion channels and transporters that contribute to ASL pH regulation. The talk will also discuss the potential of the identified targets for future therapies for CF lung disease.

Work supported by a CF Trust (UK) Strategic Research Centre Award "INOVCF" (SRC 003)

S2.3 TMEM16 proteins control mucus production and exocytosis

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Mucus in airways and intestine represents an innate defense against pathogens. Mucus traps inhaled pathogens and particles, and physically separates bacteria from epithelial cells. Proper airway mucociliary clearance and intestinal mucosal immune defense is produced by specialized goblet cells whose precise function varies depending on their location in either surface epithelium or submucosal glands in airways and intestinal crypts. In healthy lungs, mucus is formed by secretion and hydration of gel forming mucins from surface goblet cells, producing MUC5AC and MUC5B, and from submucosal glands releasing MUC5B, while the major mucin in the gut is MUC2. Although clear differences exist between airways and intestinal mucus producing cells, they may share functional properties.

Despite these protective mucus functions, intestinal pathogens and inflammatory lung diseases such as chronic obstructive lung disease (COPD), asthma, and cystic fibrosis, drive mucous metaplasia, resulting in mucin hyperproduction and hypersecretion. In the airways this often results in mucous plugging with reduced mucociliary clearance, the predominant problem in CF. A pronounced upregulation of TMEM16A-expression is observed during Th2 (IL4/IL-13) -- driven goblet cell metaplasia and mucus hypersecretion, but it is currently unclear if TMEM16A is required for mucus production or release. TMEM16A expression is driven by the IL-4/IL-13-induced transcription factor STAT6, which also induces goblet cell metaplasia together with SAM pointed domain-containing ETS transcription factor (SPDEF). As different types of inhibitors of TMEM16A blocked mucus production, we examined airway and intestinal epithelial specific TMEM16A knockout mice. Knockout of TMEM16A did not suppress basal and IL-13 induced mucus production, suggesting that other TMEM16 paralogues may control mucus production. Particle transport was enhanced and basal/constitutive mucus secretion was absent in TMEM16A-/- airways, while regulated secretion was not compromised. *In vitro* assays demonstrate regulation of exocytosis by TMEM16A, which controls intracellular Ca^{2+} signals. TMEM16 inhibitors blocked goblet cell metaplasia and mucus release, and induced relaxation of airway smooth muscles in ovalbumin-sensitized mice. Therefore TMEM16 inhibitors are proposed to improve airway function in cystic fibrosis, COPD, and asthma.

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S2.4 Role of pendrin (SLC26A4) in secretion by primary bronchial epithelial cells

Dusik Kim[1,2], Junwei Huang[1,2], Arnaud Billet[1,2], Asmahan Abuarish[1,2], Yishan Luo[1,2], Saul Frenkiel[2,3], John W. Hanrahan[1,2,3]

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Anion secretion by airway epithelial cells is essential for mucociliary clearance. Cl^- drives most fluid secretion while HCO_3^- secretion has several functions which include mucin unpacking and bacterial killing. Despite their importance in the pathogenesis of CF and other respiratory diseases, anion efflux mechanisms at the apical membrane of human airway epithelial cells remain uncertain. Pendrin is expressed in control airways and is elevated in murine models of chronic obstructive pulmonary disease and asthma. Upregulation of pendrin is also observed in vitro when primary epithelial cell cultures are treated with pro-inflammatory cytokines such as IL-4/IL-13 or IL-17A. The aim of this study was to examine the role of pendrin in anion secretion by primary human bronchial epithelial cells under control conditions and when exposed to IL-4.

Pendrin was constitutively expressed at low levels. IL-4 (10 ng ml^{-1}) treatment greatly increased (by ~112 fold) the amount of pendrin mRNA in human bronchial epithelial cells compared to untreated control cells (n=6; $p< 0.001$). Immunofluorescence staining confirmed the upregulation of pendrin by IL-4 at the protein level and localized it at the apical membrane. After pretreatment with IL-4, intracellular pH (pH_i) measurements during challenge with low Cl^- solution revealed a 4-fold increase in apical anion exchange (AE) activity in the absence of forskolin (n=8 from 2 donor). AE was not inhibited by DIDS or CFTRinh-172, however specific knockdown of pendrin using adenoviral siRNA abolished the anion exchange activity, providing further evidence that it is mediated by pendrin in IL-4 treated cells.

Interestingly, forskolin (10 mM) caused a further increase in apparent AE activity, which was also enhanced after IL-4 pretreatment and diminished in pendrin knockdown cells. pH_i and Ussing chamber measurements revealed that elevating pendrin expression had at least two distinct effects, it directly increased pendrin-mediated anion exchange and also enhanced CFTR activation by cAMP. The latter is reminiscent of regulatory protein-protein interactions between CFTR and other SLC26A transporters that have been reported previously. To test this possibility more directly, we measured whole cell anion current in BHK cells stably expressing CFTR alone or together with EGFP-pendrin. Forskolin-stimulated whole cell currents mediated by CFTR were 50% larger in cells that co-expressed pendrin (n=7-8; $p< 0.001$).

We conclude that pendrin mediates significant anion exchange and also enhances electrogenic anion flux through cAMP-activated CFTR channels when its expression in primary human bronchial epithelial cells is upregulated by IL-4.

Supported by: CF Canada, CIHR, and the Canada Foundation for Innovation.

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S2.5 Early neonatal mortality of *Slc26a9* deficient mice is triggered by airway mucus obstruction

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University of Heidelberg, Department of Translational Pulmonology, Translational Lung Research Center Heidelberg (TLRC), German Center for Lung Research (DZL), Heidelberg, Germany

Background: The epithelial Cl^- channel SLC26A9 (member of the SoLute Carrier family) has been associated with a higher susceptibility to muco-obstructive lung diseases like bronchiectasis and asthma (Bakouh N. *et al*., 2013 and Anagnostopoulou P. *et al*., 2012). SLC26A9 has been further identified as a modulator of lung function in Cystic Fibrosis (CF) patients treated with CFTR-directed therapies (Strug LJ. *et al*., 2016), supporting the role of SLC26A9 as a modifier of CF and potentially other muco-obstructive lung diseases.

Aim: The aim of this project was to determine the role of SLC26A9 during early postnatal adaptation *in vivo*.

Methods: We compared the pulmonary phenotype of wild-type (WT) and *Slc26a9* deficient (*Slc26a9*$^{-/-}$) mice on a C57BL/6 background including survival, histology, µCT imaging analysis and oxygen saturation measurements. On a functional level, transepithelial potential differences (PD) of cultured tracheal explants of WT and *Slc26a9*$^{-/-}$ mice were assessed.

Results: At birth, all genotypes were represented according to Mendelian genetics. In the first 30 minutes of life *Slc26a9*$^{-/-}$ mice presented irregular breathing resulting in a decreased survival of 48% compared to WT mice ($p< 0.05$). To determine the cause of death, histological analyses were performed and revealed mucus plugging in the trachea, proximal and distal airways in deceased *Slc26a9*$^{-/-}$ mice compared to WT mice ($p< 0.01$ for all levels analyzed). Preliminary results from µCT imaging studies support early onset airway mucus obstruction associated with atelectasis in *Slc26a9*$^{-/-}$ mice. By measuring the effect of mucus plugging on ventilation *Slc26a9*$^{-/-}$ mice showed significantly reduced oxygen saturation of 32% compared to WT mice (80%, $p< 0.01$). To evaluate the contribution of SLC26A9 Cl^- channel to the epithelial ion transport in the neonatal lung, PD was measured from cultured tracheas. Basal PD was lower in *Slc26a9*$^{-/-}$ (-18 ± 6 mV) compared to WT mice (-34 ± 6 mV; $p< 0.05$).

Conclusions: Taken together, the early phenotype is dominated by severe mortality due to mucus obstruction being associated with decreased oxygen saturation and decreased epithelial ion transport in vivo. Our data support that SLC26A9- mediated Cl^- secretion plays a critical role in airway mucus clearance in the neonatal lung and that this process is essential for normal postnatal adaptation.

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S2.6 ATP12A as an alternative therapeutic target in cystic fibrosis lung disease

Paolo Scudieri[1], Ilaria Musante[1], Emanuela Caci[2], Luis J.V. Galietta[1]

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ATP12A is a possible therapeutic target in cystic fibrosis (CF). It is expressed in the apical membrane of the airway epithelium where it is responsible for proton secretion coupled to potassium absorption. In CF epithelia, ATP12A activity, combined with the defective bicarbonate secretion due to CFTR loss of function, causes abnormal acidification of airway surface liquid (ASL) that leads to impaired antimicrobial activity and increased mucus viscosity (Shah et al., Science 2016). The importance of ATP12A and, in general, of ASL pH in CF has been questioned by a recent paper reporting that airway surface is not acidic in children with CF (Schultz et al., Nat Commun 2017).

To evaluate the role of ATP12A in CF lung disease we studied its expression and function in human airways. Immunofluorescence experiments carried out on histological sections of freshly excised non-CF bronchi revealed a very low expression of ATP12A. Instead, in CF samples, ATP12A was consistently detected on the surface epithelium, particularly on the apical membrane of non-ciliated cells and in submucosal glands. We also investigated ATP12A expression in vitro, in cultured bronchial epithelia exposed to conditions mimicking CF airways, such as treatment with bacterial components or inflammatory cytokines. Different stimuli, such as bacterial supernatant from *Pseudomonas aeruginosa* and IL-4, strongly increased ATP12A expression in both CF and non-CF cultures, suggesting that infection and inflammation may be responsible for ATP12A upregulation in CF airways.

To evaluate ATP12A function and role in the regulation of ASL pH we used a microelectrode. A small volume (75 µl) of saline solution with low buffer capacity was added to the apical side of well-differentiated CF and non-CF bronchial epithelia; after three hours, the solution was recovered and pH measured. In CF epithelia the measured pH was significantly acidic (7.3 ± 0.06) compared to that measured in non-CF (7.67 ± 0.06; $p < 0.001$). Furthermore, by adding a solution containing a higher concentration of potassium, to facilitate ATP12A-dependent proton pumping, we observed a larger ASL acidification in CF vs non-CF epithelia (deltapH = 0.4 ± 0.12 and 0.08 ± 0.05 in CF and non-CF, respectively; $p < 0.01$). Importantly, addition of ouabain, a non specific ATP12A inhibitor, reduced acidification by 50%.

Our findings confirm the acidic ASL pH in CF epithelia and support the role of ATP12A as a therapeutic target in CF. Indeed, pharmacological inhibition of ATP12-mediated proton secretion could antagonize acidification in CF airways and help to normalize mucus properties and restore antimicrobial activity.

This work was supported by Cystic Fibrosis Foundation (GALIET17G0) and by Telethon Foundation (TMLGCBX16TT).

22 March — 16:00–17:45

S03: Model systems

S3.1 Early airway disease in the CF pig model

David A. Stoltz

Lung Biology and Cystic Fibrosis Research Center, Pappajohn Biomedical Institute, University of Iowa

Loss of CFTR function leads to cystic fibrosis (CF) airway disease including airway inflammation and infection, mucus accumulation, and airflow obstruction. People with CF still experience significant morbidity, and CF remains a life-shortening disease. We chose pigs for the development of a CF model, because they are similar to humans in terms of anatomy, physiology, immune system, biochemistry, life span, size, and genetics. At birth, CF pig lungs show no evidence of inflammation in bronchoalveolar lavage fluid, histopathologic analysis, and microarray studies for inflammatory pathways. However, within weeks to months these animals spontaneously develop lung disease with features similar to human CF lung disease including bacterial infection, inflammation, mucus accumulation, airway remodeling, and airway obstruction. We have found that loss of CFTR leads to a more acidic airway surface liquid (ASL) pH in newborn CF pigs. The acidic ASL leads to impairments in at least two components of airway host defense: antimicrobial factor bacterial killing and mucociliary transport. These studies are increasing our understanding of the origins and early events of CF lung disease and will hopefully lead to the development of new therapeutics not only for CF, but potentially other airway diseases.

S3.2 Airway organoids

Jeffrey Beekman[1,2]

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New 3D culture methodologies allow long term propagation of airway organoids (AO) from biopsies or lavage fluid. AO consist of a pseudostratified epithelium containing basal cells, ciliated cells, mucus-producing goblet cells and CC-10 producing club cells. Fluid secretion phenotypes (forskolin-induced AO swelling) could be established to facilitate the analyses of CFTR-dependent ion transport and impact of CFTR modulators in CF AO. In addition, AO facilitate the contribution of non-CFTR ion channels to fluid secretion. As example, we observed that Eact1, an activator of the calcium-dependent chloride channel TMEM16A, induced similar fluid secretion as compared to corrector-potentiator restored F508del-CFTR albeit that Eact1 did not induce fluid secretion in intestinal organoids. Additionally, we developed protocols to study fluid secretion in 3D seeded organoids from conventional highly differentiated 2D airway epithelial cell cultures. These new 3D culture and assay platforms will help to study airway epithelial ion and fluid transport.

S3.3 Patient derived nasal cells, a new tool in translational research for Cystic Fibrosis?

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Institut Necker Enfants Malades. INSERM U 1151. Paris France.

Clinical studies with modulators of the Cystic Fibrosis Transmembrane conductance Regulator (CFTR) protein have demonstrated that functional restoration of the mutated CFTR can lead to substantial clinical benefit. However, studies have shown highly variable patient responses.

Conditionally Reprogrammed Human Bronchial Epithelial (HBE) cells have been used to assess the efficacy of CFTR modulators *in vitro*. However, these cells cannot be used routinely, as they are derived from explanted lungs. To overcome this problem, Human Nasal Epithelial (HNE) cell cultures have been developed. These are easy to collect by simple nasal brushing and allow to asses CFTR expression by apical membrane fluorescence semi-quantification and CFTR function by quantification of cyclic AMP (cAMP)-mediated chloride (Cl^-) transport in short-circuit-current (Isc) experiments. Recent studies showed that the response to Forskolin/IBMx and the CFTR potentiator VX-770 ($\Delta Isc_{Fsk/IBMx+VX-770}$) differentiates CFTR function between controls, healthy heterozygotes, F508del homozygotes and patients with genotypes varying from absent to residual function and correlates to in vivo Nasal Potential Difference measurements in patients sampled. This CFTR functional spectrum is related to a gradient in CFTR expression at the apical membrane which provides strong support for the physiological relevance of this evaluation.

We show that the CFTR correctors VX-809 and VX-661 significantly increase the $\Delta Isc_{Fsk/IBMx+VX-770}$ response in F508del/F508del HNE to a mean(SEM) of 23.6% (4.4) of the WT-CFTR level as well as the CFTR apical expression. Importantly, other genotypes than F508del displayed a significant correction after VX-809 or VX-661 treatment. The level of correction correlated to the basal level of CFTR activity suggesting that other causes may be considered in the level of correction, such as additional CFTR variations in cis which may affect CFTR folding or post-transcriptional modification modulating CFTR biogenesis.

Whether the *in vitro* pharmacological rescue of CFTR activity with CFTR modulators in this model is predictive of the clinical efficacy in patients, needs to be determined. In a pilot study, we show that the level of CFTR correction in HNE cultures significantly correlated with the FEV_1 variation at 6 months treated with CFTR modulators.

Such data are particularly relevant in the context of emergent therapies that rescue CFTR. This will also allow detection of patients with other genotypes than F508del that are responsive to corrector therapies. This new classification, based on "theratype" and not only genotype, is driven by patient-specific responses, thereby providing a rationale for a personalized medicine strategy tailored for every CF patient.

Gentzsch, M, *et al.* Pharmacological Rescue of Conditionally Reprogrammed Cystic Fibrosis Bronchial Epithelial Cells. Am J Respir Cell Mol Biol 2017;56:568-574.

Pranke I, *et al.* Correction of CFTR function in nasal epithelial cells from cystic fibrosis patients predicts improvement of respiratory function by CFTR modulators. Scientific Reports 2017;7:7375.

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S3.4 BMI-1 transduced basal cells as a renewable resource for air liquid interface cell culture models of cystic fibrosis

Ileana Guerini[1], Amy Walker[1], Maximillian Woodall[2], Ahmad Aldossary[1], Afroditi Avgerninou[3], Paola Bonfanti[3], Deborah Baines[2], Christopher O'Callaghan[1], Stephen Hart[1]

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Cell models of cystic fibrosis (CF) comprising air-liquid interface (ALI) cultures of primary airway cells from CF donors enable the study of many aspects of epithelial and mucociliary biology in CF as well as the evaluation of therapeutics in relevant model systems. ALI cultures are established with basal cells obtained by nasal or bronchial brushings which then differentiate into a pseudostratified epithelium. A major problem with this method is that basal cells, the progenitors of the different airway epithelial cell types, have a limited capacity for expansion requiring repeated brushing of donors. Conditionally reprogrammed cultures are one solution but have practical limitations in requiring feeder layers. We have previously described a simple alternative approach to extended passaging in which basal cells are transduced with *BMI-1,* an anti-senescence gene. In this study, we have now evaluated the utility of BMI-1-transduced basal cells as an in vitro model of cystic fibrosis.

Normal human bronchial epithelial cells (NHBE), and CF epithelial cells (CFBE) from a patient homozygous for the 3849+10kb C->T mutation, were transduced with a lentiviral vector encoding BMI-1 at multiplicities of infection (moi) of 1, 4 and 16 then maintained in submerged cell culture. Both types of BMI-1 cells maintained their rate of cell division for at least 7 passages over a 50-day period while, as expected, untransduced primary cells showed a slower growth rate after two passages. CFTR mRNA expression levels in both NHBE-BMI-1 and untransduced, primary NHBE cells (both passage 5) in well-differentiated ALI culture was approximately 1,000-fold higher than in their respective, submerged basal cell cultures as demonstrated by quantitative RT-PCR analysis. Western blot analysis of CFTR protein showed that both CFTR C and B bands were detectable in NHBE-Bmi-1 and primary cells from ALI cultures.

The CFBE-BMI-1 cells contain the 3849+10kb C->T mutation, which creates an internal intronic splice site and formation of a larger transcript, leads to complete loss of CFTR protein. This mutation, however, is associated with a mild phenotype of CF as a proportion of the transcript are correctly spliced. We confirmed by RT-PCR analysis that both primary CFBE cells on ALI (from feeder layer cultures) and CFBE-BMI-1 cells with the 3849+10kb C->T mutation showed a mixture of normal and mutant transcripts and that there was a small but detectable forskolin inducible short circuit current which was more apparent with NHBE cells. Thus, CFBE-BMI-1 cells homozygous for the 3849+10kb C->T mutation, a very rare genotype, provide, with carefully planned expansion regimes, an almost unlimited supply of cells for biological and therapeutic studies without the need to revisit the original donor.

In conclusion BMI-1 transduction provides an alternative approach to expanding epithelial basal cell cultures in CF model systems that replicate the molecular and physiological features of CF primary cells in ALI cultures.

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S3.5 Development of a robust protocol for gene-editing human pluripotent stem cells

Sara Cuevas Ocaña[1], Amy Wong[2], Magomet Aushev[3], Joey Yang[2], Neil Perkins[1], Christine Bear[4], Janet Rossant[2], Michael A. Gray[1]

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Gene-editing technologies such as TALENs and CRISPR/Cas9 have shown great promise in providing numerous knock-out/knock-in models that bring new insights to the CF field. Additionally, refined differentiation protocols of human pluripotent stem cells (hPSCs) towards several cell/tissue lineages highlight hPSCs as a valuable resource for modelling numerous multiorgan diseases, and a very powerful platform for tissue-specific drug screening in CF. Therefore, it is logical that customized gene-edited hPSC lines would greatly facilitate the improvement of our understanding of CF pathogenesis as well as drug discovery. However, the gene-editing techniques are still very challenging to perform on hPSCs, partially due to low efficiency of homology-directed repair (HDR). Therefore, our goal was to establish a robust and fast gene-editing protocol for hPSCs.

We optimized a protocol for gene-editing hPSCs by minimizing time and costs required to produce correctly edited hPSC lines. It includes: 1) highly efficient transfection method conditions, 2) rapid cell population screening for correct cleavage by designer nucleases, and 3) integration of the desired mutation/correction through HDR; 4) manual clonal isolation and rapid selective screening to identify and confirm correctly modified clones. This protocol was developed on a non-CF human embryonic stem cell (hESC) line (CA1) to produce a homozygous delF508 CF-hESC line using TALENs. Additionally, we applied this protocol to correct 3 CF-human induced (iPSC) lines homozygous for a nonsense mutation, W1282X, using CRISPR/Cas9. 1) These hPSC lines were nucleofected with plasmids encoding either *CFTR-exon11*-targeting TALENs or *CFTR-exon23*-targeting CRISPR/Cas9, in addition to a short single-strand oligonucleotide harbouring the delF508 mutation or the correct WT sequence, respectively. 2) 5-8 days post-nucleofection, the T7 Endonuclease I assay showed 16-37% cleavage at correct sites in all cell populations, suggesting TALEN and CRISPR activities in all hPSC lines. 3) Subsequently, a screening method using allele-specific PCR (AS-PCR) selective for the delF508 mutation and WT sequence was optimized for the hESC and iPSC lines, respectively, which indicated the desired sequence in all hPSC populations. 4) Clonal isolation was then manually performed and one round of clones per cell line was screened by AS-PCR, 2-6 days post-isolation. Identified positive clones were subjected to sequencing analysis which confirmed 1.9% (1/52 AS-PCR screened) pure homozygous delF508 CF-hESC line when using TALENs, in addition to 0.9% (1/110) pure heterozygous corrected, and 0.7% (1/146) and 6.4% (3/47) pure homozygous corrected iPSC lines, confirming that our optimised protocol could a produce a homozygous delF508 CF-hESC from a healthy-hESC line, and we could also correct 3 CF-iPSC lines, both in a 3-6 weeks period. These cell lines are currently being characterized in terms of their pluripotency, chromosomal stability and ability to differentiate into a specific cell lineage.

In summary, we have developed a robust and fast protocol for gene-editing hPSCs. This approach should facilitate the production of new "in-house" CF and corrected hPSC lines that will qualitatively and quantitatively expand the available range of tissue-specific models for CF pathogenesis research, and significantly improve the personalized medicine field in CF.

Supported by CF Trust-SRC003, CFIT (SickKids and CF-Canada Foundation) and Emily Foundation.

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S3.6 Nasal epithelial cells expansion and reprogramming with the anti-SMAD protocol to evaluate CFTR rescue for precision medicine

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There is an increasing availability of small molecules (potentiators, correctors, amplifiers) to rescue mutant CFTR. Such compounds are an opportunity for a personalized approach to the treatment of cystic fibrosis patients. The sensitivity of the various CFTR mutants to single molecules or combination of molecules needs to be determined to find the best treatment for each patient. This goal can be achieved by collecting nasal epithelial cells with a nasal brushing procedure. However, cells obtained by nasal brushing are mostly terminally differentiated with a limited proliferating ability. We adopted a recently published method based on the combination of three molecules DMH-1, A-83-01, and Y-27632 (Mou et al., Cell Stem Cell 19:217-231, 2016), which suppress SMAD signaling supporting the proliferation of basal stem cells. Using the anti-SMAD protocol we could generate up to 120 millions of cells from a single brushing in 4-5 passages. Such cells can be conveniently stored in frozen aliquots. When seeded on porous membranes (Snapwell inserts), cells can be reprogrammed with a specific culture medium to obtain differentiated epithelia with mucociliary properties. We estimate that 150-200 epithelia can be obtained with a single brushing. By Ussing chamber experiments, we could confirm activity of F508del-CFTR correctors, with an extent of rescue similar to that measured in bronchial epithelial cells (control: 1.3 ± 0.1 μA; VX-809: 3.4 ± 0.5 μA). Interestingly, nasal epithelial cells consistently showed a larger ENaC function compared to bronchial epithelial cells generated with the same protocol. Indeed the amiloride-sensitive current was 5.9 ± 1.3 and 0.8 ± 0.1 μA in nasal and bronchial cells, respectively. This difference may suggest an intrinsic higher ENaC expression in cells of nasal origin. In conclusion, our findings confirm the anti-SMAD protocol as a convenient method for precision medicine in cystic fibrosis. We are currently using this approach to study the pharmacological sensitivity of cells with rare mutations.

This work was supported by a grant from Telethon Foundation (TMLGCBX16TT)

22 March — 18:00–19:45

ECFS Basic Science WG Session

Cystic Fibrosis: beyond the airways

Chairs: Margarida Amaral (PT) - Jeffrey Beekman (NL)

Intestine: modelling *in vitro* intestinal organs - Sylvia Boj (NL)

Pancreas: CF-related diabetes - James Shaw (UK)

Kidney: Disturbed kidney acid/base physiology in patients with CF - Jens Leipziger (DK)

23 March — 08:45–10:30

S04: Mucus and Mucins

S4.1 Unravelling the molecular mechanisms controlling respiratory tract mucus gel formation

David J Thornton[1,2], Gareth W Hughes[1,2], Tom Jowitt[1,2], Clair Baldock[1,2], Robert Ford[2], Richard Collins[2], Michael Lockhart[1,2], Caroline Ridley[1,2]

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Polymeric mucins (MUC5AC and MUC5B) provide the structural framework of respiratory mucus. The synthesis, macromolecular assembly and ordered packaging within secretory granules of these complex glycoproteins are key events in the production of a functional mucus gel; i.e. a flowing mucus that can be transported out of the airways by the action of cilia (mucociliary clearance) and cough. Accumulation of mucus, with non-optimal transport properties, is a pathologic feature of cystic fibrosis. Whilst our understanding of mucin intracellular assembly and packaging within secretory have increased, there are still major gaps in our knowledge of these processes. For example, we have yet to establish the roles of mucin protein folded domains (N- and C-termini, and internal Cys-domains) and the highly glycosylated central mucin domains in forming the highly condensed intragranular form of polymeric mucins (1,2). Calcium and pH are important mediators of mucin supramolecular topology, in particular there is growing evidence that the mucin N-terminal protein domains promote mucin condensation via the formation of non-covalently crosslinked polymers (2,3). In this presentation, we will discuss our recent findings on the structural organisation and self-interactions of the major airway mucin MUC5B. Data will be presented from studies employing electron microscopy (single particle analysis from transmission EM and Cryo-EM), sedimentation analysis and microscale thermophoresis to investigate the highly glycosylated regions of MUC5B and recombinant MUC5B protein domains.

1. Kesimer, M. *et al., Am. J. Physiol. Lung Cell Mol. Physiol.* (2010) 298, L15-22.

2. Ridley, C. *et al., J Biol Chem.* (2014) 289, 16409-420.

3. Ambort, D. *et al., Proc. Natl. Acad. Sci. U.S.A.* (2012) 109, 5645-50.

S4.2 Reporter Muc5b-GFP mouse to study Muc5b functions

Valérie Gouyer, Frédéric Fottrand, Jean-Luc Desseyn

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Altered mucus properties, which is an hallmark of cystic fibosis (CF), greatly impair mucociliary transport. MUC5B and MUC5AC are the two main macromolecules of respiratory mucus. They are released as long polymers and form the mucus hydrogel in contact of water in the lumen. In human, the MUC5AC gel-forming mucin is produced by airway goblet cells at the epithelium surface and *MUC5B* in submucosal gland mucous cells and also goblet cells in many respiratory diseases. In mouse, Muc5b is primarily produced by club cells in the lungs and submucosal gland mucous cells of the trachea. Null mice for Muc5ac and Muc5b suggested that the two mucins have different functions with Muc5b being critical for mucociliary clearance [1]. However, functions of the two mucins in health and disease is still poorly understood.

To assess the biological function of Muc5b in physiological condition and in CF, we engineered by homologous recombination a reporter Muc5b mouse where the unique Stop codon of the *Muc5b* gene was replace in frame with a GFP tag sequence [2,3]. In addition, the model was designed to genetically access to a new conditional knock-out model by Cre-mediated excising the last two exons of *Muc5b*.

Muc5b expression profile was assessed by immunohistochemistry, fluorescence in fresh tissues and in living mice using confocal laser endomicroscopy. Because adult Muc5b-deficient mice show altered pulmonary function leading to respiratory failure, we have examined the microflora from bronchoalveolar lavage of Muc5b-deficient mice in comparison to Muc5b-GFP mice. Abnormalities of the respiratory tract of Muc5b-deficient mice currently under investigation seem highly similar to the one reported in human subjects with CF and CF animal models and will be presented. Future directions to study Muc5b in CF using our mouse reporter model will be discussed.

1.Roy MG *et al*. (2014) *Nature* 505:412

2. Portal *et al.* (2017) *Sci. Rep.* 15;7:44591

3. Portal *et al*. (2017) *PLoS One* 29;12(3):e0174764

S4.3 The biophysics of airway mucus in health and disease

Brian Button[1,2]

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The pulmonary mucus clearance system represents a key innate host defense system that protects the lung from inhaled pathogens and noxious chemicals. However, failure of mucus clearance represents an important contributor to disease progression in many muco-obstructive lung diseases, including cystic fibrosis (CF). Despite many years of research, the mechanisms that produce mucostasis in these diseases remain elusive. The goal of this talk is to present our recent work on how mucus and mucins (the large viscoelastic glycoproteins in mucus) are organized to ensure efficient clearance in health and how alterations in their biochemical and biophysical properties result in a loss of mucus clearance. Key to our understanding of this system is the observation that in health, epithelial ion/water transport and mucin secretion rates are finely regulated to produce a well-hydrated mucus layer (98% water/2% solids) that is efficiently cleared from the lung by the action of cilia beating and coughing. However, in CF, defects in ion transport, coupled with increases in mucin secretion, result in a mucus which can exceed 15% solids. We have previously shown that such an increase in mucus concentration is associated with an increase in the mucus osmotic pressure. Importantly, when the osmotic pressure of the mucus layer exceeds that of the periciliary layer (PCL), water is removed from the PCL space, producing osmotic compression of the cilial layer and eventual failure of cilia-mediated clearance.

In addition to defects cilia-mediated clearance, persons with CF also exhibit reduced cough clearance, a mechanism intended to augment the clearance of thickened, accumulated, mucus from the airways. We hypothesized that in CF, mucus is harder to clear by cough as a consequence of increased cohesive (mucin-mucin) and adhesive (mucin-cell surface) interactions. Indeed, our novel studies presented here reveal that the hyperconcentrated mucus associated with CF is more adherent to the epithelial cell surface, making it hard to remove by airflow associated with cough. In addition to changes in mucus concentration, our studies also support the notion that mucus with higher concentration of MUC5AC mucin is harder to clear than a MUC5B-dominated mucus, consistent with previous reports of reduced mucus clearance in MUC5B knockout mice. Finally, from a therapeutics perspective, we have identified a number of putative therapeutic agents, including mucus hydrators and mucolytics (such as reducing agents), which can reduce these interactions and potentially improve/restore cilia- and cough-mediated mucus clearance in the airways of CF patients.

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S4.4 Intramolecular interactions of the airway mucin MUC5B and their role in packaging within secretory granules

Caroline Ridley, Richard F. Collins, Tom A. Jowitt, Clair Baldock, David J. Thornton

University of Manchester, Wellcome Trust Centre for Cell-Matrix Research, Faculty of Biology, Medicine and Health, Manchester Academic Health Science Centre, Manchester, United Kingdom

Mucus plays an essential role in forming a barrier to protect the respiratory epithelium from pathogenic and environmental challenges. MUC5B and MUC5AC are the major polymeric, gel-forming mucins in respiratory mucus and are primary determinants of airway mucus properties. MUC5B is stored in the secretory granules of mucous cells, and to a lesser extent in goblet cells, prior to secretion onto the epithelial surface to form mucus. Inside the secretory granule, MUC5B is stored in a dehydrated and compact form; compaction is driven by acidic pH and calcium-rich environment and mediated by calcium-dependent, non-covalent interactions between N-terminal domains of MUC5B (1). After secretion, the increased water availability, neutral pH and lower calcium concentration result in rapid expansion of linear MUC5B polymers via the uncoupling of the interactions between N-terminal domains. The transition from the compact to expanded linear form of MUC5B is vital for the correct rheological properties of the mucus gel and effective mucociliary clearance. In the CF lung, changes in the extracellular milieu (decreased water availability, and increased pH and calcium concentration) contribute to abnormal mucin expansion and conformation, yielding mucus with aberrant transport properties which leads to mucus accumulation and blockage of small airways and recurrent infection (2).

Our objective in this study was to investigate whether other intramolecular interactions between protein domains of MUC5B are active during mucin packaging in secretory granules. Therefore, we have investigated intramolecular interactions between the N- and C-terminal protein domains of MUC5B. The complete N-terminus (D1-D2-D'-D3) and C-terminus (D4-B-C-CK) of MUC5B were expressed in mammalian cells. Recombinant C-terminal MUC5B protein was analysed using multi-angle laser light scattering, analytical centrifugation, small-angle X-ray scattering and transmission electron microscopy. Interactions between N- and C-terminal protein domains were determined using surface plasmon resonance and micro-scale thermophoresis. Structural analysis of the C-terminal dimer of MUC5B identified a rod-like structure, with a flexible region and two globular domains. No homotypic interactions were detected between C-terminal regions. However, results showed that at pH 6.0 in the presence of calcium, the N-terminal and C-terminal of MUC5B formed heterotypic complexes, which were dissociated after calcium chelation by EGTA and increase of pH. These results highlight novel intramolecular interactions between MUC5B protein domains that contribute to the organisation of the mucin molecule during packaging within secretory granules.

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1. Ridley, C., *et al*., *Assembly of the respiratory mucin MUC5B: a new model for a gel-forming mucin.* J Biol Chem, 2014. **289**(23): p. 16409-20.

2. Boucher, R.C., *New concepts of the pathogenesis of cystic fibrosis lung disease.* Eur Respir J, 2004. **23**(1): p. 146-58.

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S4.5 Identifying pathways regulating goblet cell metaplasia: phenotypic screening with bronchospheres

Henry Danahay[1], Clive McCarthy[1], Roy Fox[2], Martin Gosling[1]

[1]Enterprise Therapeutics, Brighton, United Kingdom, [2]University of Sussex, Sussex Drug Discovery Centre, Brighton, United Kingdom

The composition of mucus in the CF airway, and in particular the hydration status, significantly affects its clearance and thereby the potential for form plugs, restrict airflow and create a nidus for chronic microbial colonisation. A variety of largely ion channel-based strategies are being employed to promote mucosal hydration e.g. CFTR repair, ENaC blockers and TMEM16A potentiators. An alternative, but complimentary approach would be to reduce the excessive production and secretion of the mucin proteins that contribute to the solids component of the mucus gel.

One approach to reduce excessive mucus production is to reduce the number of mucus producing goblet cells in the airways. To identify drug targets that could be regulated to achieve this, we have utilised a 3D culture model of the human airway epithelium, 'bronchospheres'. Bronchospheres are derived from primary human airway basal cells, and can be cultured to form a well-differentiated mucociliary epithelium without the need for an air-liquid interface. Bronchospheres are cultured in a 384 well assay format that makes them amenable to medium throughput compound screening. Treating bronchospheres with inflammatory mediators such as IL-13 induces a mucus hypersecretory phenotype with increased numbers of goblet cells and reduced numbers of ciliated cells. Our hypothesis was that the co-administration of test compounds with IL-13 would identify compounds capable of preventing goblet cell formation with the opportunity to seed future drug discovery programs.

Bronchospheres were cultured as previously described (Danahay et al., Cell Rep. (2015) 10(2):239). On day 2 after seeding primary human airway basal cells, treatment with IL-13 ± test compounds was initiated. On day 8, media and treatments were topped-up and on day 14 bronchospheres were lysed and RNA isolated. QPCR was then used to assess the expression of cell-specific markers: MUC5AC (goblet cells) and FOXJ1 (ciliated cells). Compounds that induced a ≥2-fold reduction in expression of MUC5AC were classified as hits. This hit list was then refined by checking the expression of FOXJ1. Compounds that had likewise attenuated FOXJ1 expression by ≥2-fold were deprioritised as these likely represented a non-specific effect on epithelial differentiation. Compounds that either maintained or enhanced FOXJ1 expression in addition to repressing MUC5AC gene expression were prioritised for validation using traditional air-liquid interface cultures.

In total, 92 hit compounds from the bronchosphere screen were tested for effects on goblet and ciliated cell numbers in ALI HBE cultures using quantitative immunohistochemistry. Of these, 38 (41%) significantly attenuated the MUC5AC+ stained area in IL-13 treated HBE and either maintained or increased the FOXJ1+ stained area. Validated hits were then aligned based on their previously reported pharmacological activity to enable common pathways to be identified and to refine our hypotheses through further exemplification of pathway regulators.

Based on this screen, we have identified pathways that regulate the induction and maintenance of a goblet cell metaplasia in vitro and are progressing a lead optimisation program for eventual therapy in respiratory diseases associated with mucus obstruction.

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S4.6 Lack of CFTR results in impaired innate immunity in small airways

Joseph Zabner

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It is often assumed that cystic fibrosis (CF) lung disease begins in the small airways. While a number of observations suggest this assumption is correct, we do not have direct experimental evidence. Because we have more knowledge of host defense defects in large CF airways, we might conjecture that the abnormalities in small CF airways are the same. However, differences in epithelial morphology, cell types and gene expression profile, and lack of submucosal glands and continuous cartilages suggest that it is not safe to assume that small airways are simply "small" large airways. Our preliminary data show that newborn CF small airways are more acidic than non-CF, and have an impairment in host defenses. Bacterial killing by airway surface liquid is impaired compared to non-CF, and mucociliary transport is altered in CF small airways after stimulation of goblet cell secretion with ATP. In large airways CFTR and ATP12A regulate ASL pH and innate immunity. We found that small airways do not express ATP12A, instead they seem to rely on V-ATPase for H^+ secretion. We found that V-ATPase is expressed on the apical surface of a specific cell type of small airway epithelial cells. An isoform of the ATP6V0D subunit, ATP6V0D2, is required for apical localization of V-ATPase in the small airways, and interventions can regulate the translocation of V-ATPase to the apical membrane. Understanding the contribution of small airways to disease and in identifying strategies for better treatments/cures of CF. It will also educate us about small airways physiology and how it may be abnormal in other diseases.

23 March — 11:00–12:45

S05: Inflammation

S5.1 Mechanisms of neutrophil activation in early cystic fibrosis airway disease

Camilla Margaroli[1,2], Luke W Garratt[2], Hamed Horati[3], Joshua D Chandler[1,2], Lokesh Guglani[1], Anthony Kicic[3], Harm AWM Tiddens[4], Balazs Rada[4], Limin Peng[5], Bob J Scholte[4], Stephen M Stick[3], Hettie M Janssens[4], Rabindra Tirouvanziam[1,2]

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Background: Neutrophils are the most abundant leukocyte subset in human blood. Due to their hypercondensed chromatin and short lifespan, neutrophils are generally thought to function along preprogrammed paths, such as phagocytosis and intracellular killing of pathogens, or formation of neutrophil extracellular traps (NETosis), rapidly followed by cell death. In adult CF patients, neutrophils are continuously recruited to the airway lumen, and high-content phenotyping by our group revealed that they undergo pathological conditioning therein, leading to enhanced metabolism and survival, transcriptional burst, and other functional adaptations perpetuating inflammation. A key question is whether similar pathological conditioning of neutrophils occurs in the airway of CF infants, at an early stage of the disease.

Methods: We investigated airway disease in CF and control infants aged 3 months to 6 years enrolled at our three institutions. Complete clinical workup of patients is performed, including computed tomography (CT) to monitor early airway damage. Blood and airway samples are assessed by high-content molecular (proteomics, lipidomics, metabolomics), and cellular (genomics, cytometry) methods to identify putative mechanisms of neutrophil activation and inflammation *in vivo*. These mechanisms and relevant drugs are further assessed *in vitro*, using an organotypic model of neutrophil transepithelial recruitment and pathological conditioning by airway fluid derived from patients.

Results: CF infants as early as 3 months of age display pathological conditioning of neutrophils, featuring active degranulation of elastase- and myeloperoxidase-rich granules, and the presence of molecular markers of neutrophil dysfunction, some of which track with airway damage measured by CT. Moreover, airway fluid from CF infants induces the transepithelial recruitment and pathological conditioning of neutrophils *in vitro*. Because pathological conditioning of CF airway neutrophils readily occurs within the first months of life, and because airway fluid from CF infants triggers pathological conditioning of transmigrated blood neutrophils from healthy controls, we posit that the CF airway environment, likely due to abnormal epithelial signaling (cf. abstract by Scholte et al.) is intrinsically defective, and leads recruited neutrophils to undergo abnormal activation, prior to the advent of chronic infection. NETosis was also measurable in some of the infant samples. Interestingly, our cell phenotyping data point to dysfunction of resident macrophages as a potential process through which recruited neutrophils assert their dominance over the CF milieu.

Conclusions & perspectives: Neutrophils play a key role in CF airway pathogenesis at a very early stage. High-content molecular and cellular phenotyping methods can yield novel biomarkers of early CF disease, and inform on the relevance, and benefits of therapeutic approaches targeting processes inherent to CF airway neutrophils such as metabolic licensing, degranulation, and transcriptional burst. These questions will be addressed in upcoming prospective and interventional longitudinal studies conducted by our multinational consortium.

Funding: We gratefully acknowledge support from the Center for CF and Airways Disease Research in Atlanta (to CM, JDC, LG, RT), the Australian NHMRC 1142505 (to LWG, AK, SMS, RT), the US CF Foundation TIROUV17P0 (to CM, RT), and the US NIH R01HL126603 (to HH, HAWMT, LP, BJS, HMJ, RT).

S5.2 Bacteria-driven peribronchial lymphoid neogenesis in cystic fibrosis and bronchiectasis

Pierre-Régis Burgel

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Tertiary Lymphoid Structures (TLS) are ectopic structures whose architecture is closely similar to that of secondary lymphoid structures (SLO) (spleen, lymph nodes). TLS were first depicted as lymphoid aggregates called bronchus-associated lymphoid tissue (BALT). Although these structures are absent in normal human lungs, TLS have been described in several lung diseases (e.g., lung cancer, idiopathic pulmonary fibrosis). Our group recently examined the presence of TLS in bronchiectasis and in cystic fibrosis (CF) lungs and described the presence of numerous peribronchial TLS containing B lymphocytes, T lymphocytes, germinal centers and high endothelial venules that were immediately adjacent to the airway epithelium.

Next, we examined the role of persistent bacterial infection in the formation of TLS (a process called lymphoid neogenesis) by studying C57/BL6 mice after a single intratracheal instillation of agarose beads, comparing sterile beads and beads containing live P. aeruginosa (PA)- or S. aureus (SA). Although mouse lungs contained no lymphoid aggregate at baseline, persistent PA- or SA-airway infection induced peribronchial lymphoid neogenesis within 2 weeks. At day 14 after instillation, lymphoid aggregates expressed markers of tertiary lymphoid organs and the chemokines CXCL12 and CXCL13. The airway epithelium was an important site of CXCL12, CXCL13 and IL17A expression, which began at day 1 after instillation.

These initial data showed that peribronchial tertiary lymphoid organs are present in bronchiectasis and in CF, and that persistent bacterial infection triggered peribronchial lymphoid neogenesis in mice. Peribronchial localization of tertiary lymphoid organs and epithelial expression of chemokines suggested roles for airway epithelium in lymphoid neogenesis.

This work is currently followed by experiments aimed at describing (1) possible roles of lymphoid follicles in host defense vs. autoimmunity (2) examining the roles of B and T cells during persistent bacterial infection in mice.

S5.3 Role of IL-1 receptor signaling in the pathogenesis and as potential therapeutic target in CF airway inflammation

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In many organs including the heart and the brain, hypoxic cell death provides a strong trigger for sterile neutrophilic inflammation via Interleukin-1 receptor (IL-1R) signaling (1). Although regional hypoxia due to mucus plugging is a common feature in the airways from patients with cystic fibrosis (CF) (2), its role in the pathogenesis of neutrophilic inflammation has received little attention. In previous studies, we observed that airway mucus obstruction causes hypoxic epithelial necrosis that is associated with the onset of neutrophilic airway inflammation in βENaC-overexpressing (βENaC-Tg) mice with CF-like lung disease (3). To elucidate the role of hypoxic epithelial necrosis and IL-1R signaling in the development of neutrophilic airway inflammation and structural lung damage in CF lung disease, we used genetic deletion and pharmacological inhibition of IL-1R in βENaC-Tg mice and determined effects on airway epithelial necrosis, levels of inflammatory markers (IL-1α, KC and neutrophils) in bronchoalveolar lavage, structural lung damage and mortality. Further, we analyzed lung tissues from patients with CF and controls for the presence of airway mucus plugging and epithelial necrosis. These studies showed that lack of IL-1R had no effect on epithelial necrosis and elevated IL-1α released from dying cells, but reduced airway neutrophilia, structural lung damage and mortality in βENaC-Tg mice (4). Treatment of adult βENaC-Tg mice with the IL-1R antagonist anakinra had similar protective effects and reduced neutrophilic inflammation and structural lung damage. Further, we found that the numbers of necrotic airway epithelial cells were elevated and correlated with small airways mucus obstruction in patients with CF (4). These results spurred a recent genetic association study supporting that the *IL1R* gene is a modifier in CF (5). Collectively, these results support an important role of of the IL-1R signalling pathway, either activated by IL-1α released from dying hypoxic cells in mucus-obstructed airways or by IL-1β induced by bacterial infection, in the pathogenesis of chronic neutrophilic inflammation causing progressive lung damage in patients with CF. These findings from genetic and functional studies also indicate that inhibition of IL-1R signaling may be a promising anti-inflammatory strategy in CF and potentially other lung diseases associated with chronic airway mucus obstruction and neutrophilic inflammation.

Supported in part by the German Ministry for Education and Research (FKZ 82DZL00401 and FKZ 82DZL004A1).

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S5.4 L iminosugars: new anti-inflammatory drugs for CF lung disease?

Daniele D'alonzo[1], Annalisa Guaragna[1], Slvia Munari[2], Nicoletta Loberto[3], Alessandra Santangelo[2], Ilaria Lampronti[4], Anna Tamanini[2], Alice Rossi[5], Serena Ranucci[5], Ida De Fino[5], Alessandra Bragonzi[5], Massimo Aureli[3], Aessandro Sonnino[3], Giuseppe Lippi[2], Roberto Gambari[4], Giulio Cabrini[2], Govanni Palumbo[1], Maria Cristina Dechecchi[2]

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Novel drugs tailored to CF lung pathology able to reduce the tissue damage due to the excessive inflammation in CF patients are still an unmet need. Protective inflammatory response during airway infection is mediated by sphingolipids (SLs) (Sharma L and Prakash H, 2017), therefore SL metabolism could be modified to benefit host defense. In this regard, several inhibitors have been proposed. The iminosugar N-butyl d-deoxynojirimycin (d-NBDNJ, Miglustat) produces an anti-inflammatory effect in CF bronchial cells, by targeting β-glucosidase 2 (GBA2) (Loberto N, 2014). Iminosugars are attractive carbohydrate mimics and represent lead compounds for developing new drugs for many diseases (Horne G, 2011). The high potential of l-iminosugars as novel drug candidates has been recently proposed (D'Alonzo, 2017). Interestingly, l-iminosugars have demonstrated to be able to efficiently recognize and inhibit d-glycosidases (Horne, 2011). To better exploit the benefits of iminosugars, we focused on l-enantiomers and on racemic mixtures composed by d- and l-enantiomers. In order to identify similar chemical compounds able to reduce the inflammatory response to *P.aeruginosa* at low dosage, the effects of these compounds in CF bronchial cells and murine models of lung infection were studied. Nanomolar concentrations of monovalent and multivalent DNJ iminosugars and racemic mixtures of N-alkylated iminosugars produced an anti-inflammatory effect in CF bronchial cells. Combination of low doses of miglustat and its l-enantiomer was more potent than miglustat alone and reduced the inflammatory response to *P.aeruginosa* also in CF primary cells. Analysis of d- and l-iminosugars demonstrated that l-enantiomers were more specific than the corresponding d-isomer for GBA2. No toxicity was observed both *in vitro* and *in vivo*. Combination of low doses (10 mg/Kg) of miglustat and its l-enantiomer administered to C57Bl/6NCr mice 24 hours before bacterial challenge improved the resistance to infection. Very importantly, treatment with l- but not d-enantiomer alone significantly reduced the amount of neutrophils recruited in bronchoalveolar lavage without increasing the bacterial load. These results confirm that DNJ-containing iminosugars are effective in reducing the inflammatory response to *P.aeruginosa* in CF bronchial cells. Our novel findings *in vivo* give a new insight into therapeutic approaches for managing respiratory infections by using N-alkylated l-iminosugars that can be regarded to improve effectiveness even at low dosage and to address selectively more specific targets.

Funding: This research was supported by the Italian Cystic Fibrosis Research Foundation grant FFC #22/2015 to MCD and MA.

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S5.5 ENaC has a pivotal role in modulating autoinflammation and glycolysis in cystic fibrosis

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Background: Cystic fibrosis (CF), the most common genetic disorder in European populations, is a multi-system disorder affecting the digestive, endocrine and respiratory systems. The cystic fibrosis transmembrane conductance regulator (CFTR) not only acts as a chloride ion channel but is also an inhibitor of the epithelial sodium channel (ENaC). When absent, both functions are lost resulting in reduced chloride and increased sodium flux. The CF lung is highly inflammatory with elevated innate immune cell infiltrations, associated with high levels of IL-1b. Metabolism and inflammation are inextricably linked, with both systems impacting on each other. This study aimed at understanding the relationship between increased sodium flux with metabolism and NLRP3 inflammasome signalling in CF.

Methods: Human bronchial epithelial cell (HBEC) lines, with various classes of CFTR mutation, were studied. Blood samples were taken from patients with stable CF, active CF, autoinflammatory patients, non-CF bronchiectasis (NCFB) and healthy controls (HC) for monocyte and serum studies. ELISAs were performed for IL-1b, IL-18, IL-1ra, ATP, glucose, succinate and lactate. A Seahorse extracellular flux analyser measured oxygen consumption and proton production. Flow cytometry was used to detect M1-type and M2-type macrophages that were previously polarised *in vitro*, as well as ASC specks. Fluorescent probes for sodium and potassium were used to measure ion flux *in vitro.* Amiloride, lipopolysaccharide (LPS), ATP, 2-Deoxy-D-glucose (2-DG) and MCC950 (NLRP3 inhibitor), were used to treat cells *in vitro*.

Results: Serum levels of IL-1b, IL-18, IL-1Ra and ASC specks were elevated in CF samples compared to HC and patients with NCFB. When monocytes were isolated and polarised to macrophages, there were significantly less anti-inflammatory M2-type macrophages from patients with CF with decreased IL-10 and increased IL-6 secretion. CF monocytes and HBECs had elevated sodium influx upon ATP stimulation and a consequential drop in intracellular potassium levels, a known activator of the NLRP3 inflammasome. Due to the fact that the activity of the sodium/potassium ATP-gated channel is elevated in CF to compensate for increased sodium influx, we measured cellular metabolism to understand how this greater energy demand impacts on glycolysis. ATP, glucose, succinate and lactate were all elevated in CF monocytes and HBECs. Finally, we stimulated monocytes and HBECs with LPS and ATP to activate the NLRP3 inflammasome. We found that monocytes and HBECs from patients with CF hyper-responsive to NLRP3 inflammasome activation and inhibiting ENAC with amiloride reduces this hyper-responsiveness.

Conclusions: Collectively, our findings reveal novel intrinsic mechanisms behind the excessive degrees of inflammation, independent of infection, that are observed in CF. We have shown that IL-1b and IL-18 are dominant in human CF and drive the systemic disease. We demonstrate that CF monocytes and macrophages are skewed towards a proinflammatory phenotype and that increased Na+ influx, via ENaC, contributes to metabolic reprogramming and NLRP3 activation in primary cells from patients with CF and also in cell lines, containing CF-associated mutations. Finally, we show that amiloride, a short-acting small-molecule ENaC inhibitor, is capable of regulating these proinflammatory and metabolic perturbations of the innate immune response in vitro.

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S5.6 Complex network involving miRNA and RNA-BP in CF

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Background: Cystic fibrosis (CF), the most common lethal rare disease, is characterized by lung inflammation. Changes in miRNAs expression profile in CF cells have been described in the literature, mainly by quantification of a part of the miRNA pool (TLDA system).

Aims: In order to obtain an overview, we decided to use an unbiased sequencing approach (Illumina) to establish profiling of miRNA expression. First, we defined the genes and/or signalling pathways targeted by these deregulated miRNAs. Secondly, we focused on an anti-inflammatory protein, named TTP and studied its regulation and impact on its targets mRNAs.

Materials and methods: RNAs or proteins were extracted from CF bronchial cell lines or from *ex vivo* reconstituted epithelium cultured in Air-Liquid Interface, ALI (5 non-CF and 5 CF). To assess miRNAs and TTP role, expression level of miRNAs, transcripts and proteins of interest was evaluated by RT-qPCR or immunoblot, respectively. To confirm TTP binding on *CFTR*-3'UTR RNA pull-down assays were also carried out.

Results: In CF ALI cultures, we showed the deregulation of 35 miRNAs, which mainly target mRNAs involved in PI3K-Akt pathway (21/35) and MAPK pathway (18/35). To validate the role of these genes in CF, we quantified the expression of more than 200 genes by qPCR and specific arrays, and confirmed the deregulation of more than 30 genes. In addition, search for a crosstalk between PI3K-Akt and MAPK pathways pointed out RNA-BPs that control gene expression of several genes. As a good candidate, we next focused on the TTP protein, a RNA-BP targeted by 6 deregulated miRNAs and deregulated in CF cells. The TTP non-phosphorylated form modulates negatively the stability of many interacting partners including pro-inflammatory proteins, such as IL-8. We showed by immunoblot an accumulation of both the TTP phosphorylated form in CF cells (inactive form) and the MK2 phosphorylated form, responsible for TTP inactivation. By using drugs we showed that MK2 protein, typically activated through MAPK pathway, is phosphorylated by Erk pathway in CF cells. Next, we searched for TTP's targets and *in silico* analysis (RBPDB database) predicted the presence of binding sites on the 3'UTR of *CFTR* mRNA. Occupancy of TTP on *CFTR* mRNA was validated by RNA pull-down. Overexpression and silencing of TTP showed a positive effect on *CFTR* mRNA. Impact on other targets are being tested.

Conclusion: miRNAs studies allowed us to highlight a new regulation pathway in CF cells, involving an anti-inflammatory protein. Decoding miRNAs/TTP partners' map could be of great interest in CF.

Acknowledgements: Supported by Vaincre La Mucoviscidose

24 March — 08:45–10:30

S06: Host-pathogen interactions

S6.1 Anaerobic bacteria in Cystic Fibrosis pulmonary infection

Michael M Tunney

Although Cystic Fibrosis (CF) is a multi-system disease, chronic bacterial pulmonary infection leading to an irreversible decline in lung function is the main cause of mortality and morbidity. Routine bacterial culture methods identify the aerobic bacteria *Pseudomonas aeruginosa, Staphylococcus aureus* and *Burkholderia cepacia* complex as the principle pathogenic bacteria in adult CF pulmonary infection. However, using enhanced culture and cultivation-independent techniques, we have shown that anaerobic bacteria are detected in respiratory samples provided by people with CF of all ages and stages of disease suggesting that they are common and persistent members of the lower airway community. The most frequently identified anaerobic bacteria in the lower airways, *Prevotella, Veillonella* and *Actinomyces* are common to both CF and healthy lungs. The relative abundance of anaerobes in the lower airways fluctuates over time with reduced lung function and increased inflammation associated with a decreased anaerobic load. Therefore, the role of anaerobic bacteria in the CF lower airways might be as members of a normal airway microbiota with disruption of this microbiota and dominance of recognised pathogens responsible for disease progression. However, some of the anaerobic bacteria detected, e.g. *Prevotella*, are resistant to antibiotic treatment, produce virulence factors and may enhance pathogenicity and colonisation by recognised CF pathogens. This may indirectly contribute to increased resistance in the CF microbiota and antibiotic treatment failure at different stages of respiratory disease. Further studies are required to understand the activities and complex interactions of microbes in early-stage disease and how the evolution of the bacterial community towards a less diverse end-stage disease can be prevented

S6.2 CF airways metagenome

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High-throughput untargeted metagenome sequencing provides in-depth information about the composition of the microbial communities of viruses, bacteria, archaea and unicellular eukaryotes in the habitat of interest. We analyzed the microbial metagenome in nasal lavages, oropharyngeal swabs and induced sputa collected on one to seven occasions within a period of up to 25 months from 47 exocrine pancreatic insufficient (PI) and 27 exocrine pancreatic sufficient (PS) individuals with CF. The distribution of body mass index and of FEV1 of the study cohort matched with the age- and gender-corrected centile distribution of the 2013 patient registry of the European CF Foundation implying that the study cohort is representative for the disease severity of the current CF patient population.

Genomic DNA isolated from the microbiological specimens was subjected to shotgun metagenome sequencing. After processing, trimming and quality filtering, the primary reads were mapped onto the human reference genome and a microbial pangenome of about 8.000 completely sequenced genomes. After normalization by genome size and by G+C contents of the read and of the respective species, the normalized read numbers were deposited in the database representing the absolute abundance of bacterial species and higher taxonomic ranks.

Deep metagenome sequencing typically identified about a dozen DNA viruses and fungi and several hundred bacterial taxa in samples from children, adolescents and adults. The oral communities of the CF hosts lacked typical CF pathogens, but the upper and lower airways exhibited a CF-typical signature of microbes dominated by Firmicutes in samples from PS patients and Proteobacteria in samples from PI patients. A rather normal microbial flora of oral organisms was rather frequent in the airways of PS patients, but uncommon in those from PI patients suggesting that the residual function of mutant CFTR is the major modifier of the CF airways microbiome. Correspondingly the levels of neutrophilic enzymes and proinflammatory interleukins showed associations with the bacterial airway microbiome composition in PI, but not in PS hosts. The airway samples could be assigned to four major groups of CF bacterial microbiomes led by either one of the typical CF pathogens *Staphylococcus aureus, Pseudomonas aeruginosa* or *Haemophilus influenzae* or led by microaerophilic organisms (Rothia, Prevotella, Streptococci), respectively. Two subgroups of *P. aeruginosa*- and/or *S. aureus-negative* bacterial metagenomes were identified, one dominated by *Rothia* and *Prevotella* spp. and the other dominated by oral streptococci other than the well studied *S. pneumoniae / S. oralis* clade, i.e. *S. parasanguinis* and *S. salivarius*. High abundance of the latter two species was preferentially observed in individuals with normal spirometry. In low-diversity metagenomes the *S. aureus* and *P. aeruginosa* communities typically consisted of minor and of one to two major clone types exhibiting substantial within-clone variation of the core and accessory genome. The rare clones and clonal variants constitute a low copy genetic resource which could rapidly expand as a response to habitat alterations such as antimicrobial chemotherapy or invasion of novel microbes.

S6.3 Invasive sampling of the lungs of children with CF suggests a revised model of CF infection pathogenesis

Pradeep Singh

University of Washington

Programme note: the details are not authorised for inclusion

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S6.4 Intracellular persistence of *Pseudomonas aeruginosa* in airway epithelial cells and CF lungs

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Cystic Fibrosis (CF) lung disease is characterized by chronic *Pseudomonas aeruginosa* infections in the CF airways. Why *P. aeruginosa* persists despite antimicrobial treatments and activation of innate immune responses remains incompletely understood. To date, the prevailing view suggests that impaired mucocilliary clearance and *P. aeruginosa*'s biofilm lifestyle are the primary mechanisms contributing to the persistence and failed eradication of *P. aeruginosa* in the CF lung. Although *P. aeruginosa* is primarily known as an extracellular pathogen, previous studies have observed that *P. aeruginosa*'s can be internalized by epithelial cells *in vitro*. What the biological relevance of this process and whether it occurs in the CF lung remains largely unknown. This led us to hypothesize that *P. aeruginosa* can be internalized and persist in airway epithelial cells, and that this unrecognized intracellular lifestyle contributes to *P. aeruginosa*'s evasion of extracellular antibacterial drugs and host defenses.

We have developed a novel *in vitro* model of long-term intracellular persistence of *P. aeruginosa* in human airway epithelial cells using several cell lines (Beas-2B, CBFE-WT and CFBEΔF508), and have tested different *P. aeruginosa* strains, including the reference strain PAO1, mucoid and non- mucoid CF clinical isolates, and several genetically engineered mutants deficient in T3SS, flagellar motility among others. We observed that *P. aeruginosa* persists and replicates in B2B and CFBE cells over 5 days without activating intracellular inflammatory signaling pathways, or causing significant host cell cytotoxicity and death. Approximately 5 to 10% of the initial bacterial inoculum is internalized, and the intracellular bacterial burden increases 2-fold over 5 days. Moreover, intracellular *P. aeruginosa* is increased ~ 2-fold in CFBEΔF508 cells compared to CFBE-WT cells, and this is reversed with VX-770/VX-809. Loss of flagellin promotes *P. aeruginosa* persistence in airway epithelial cells, while no difference is observed between T3SS(+) and T3SS(-) strains.

In order to determine whether the CF lung harbor intracellular *P. aeruginosa*, we examined lung tissues from CF lung explants. Preliminary semi-quantitative microbiological analyses and 3D confocal microscopy imaging by MiPACT (Microbial Identification Passive CLARITY Technique) coupled with in situ localization of *P. aeruginosa* within lung tissues by immunofluorescence and hybridization chain reaction, suggest the presence of intracellular *P. aeruginosa* in lung epithelial cells.

In summary, our *in-vitro* data suggests that *P. aeruginosa* can be internalized and persists intracellularly within human airway epithelial cell cultures. Preliminary results also suggest that CF lung explants harbour intracellular *P. aeruginosa* within lung epithelial cells. These findings raise the possibility that *P. aeruginosa* may have an unrecognized intracellular reservoir within the CF lung and highlight a potentially new mechanism of bacterial persistence.

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S6.5 Airway surface liquid acidification initiates host defense abnormalities in Cystic Fibrosis

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Background: Cystic fibrosis (CF) is a lethal autosomal recessive genetic disorder caused by mutations in the *CFTR* (Cystic Fibrosis Transmembrane conductance Regulator) gene encoding the CFTR protein, an anionic channel, secreting chloride (Cl^-) and bicarbonate (HCO_3^-). Most of the patients are homozygous for the F508del mutation. The major cause of morbidity is infection of the airways which starts from the first hours of life, mainly involving *Staphylococcus aureus*. Data in the CF pig model established a relationship between an acidic airway surface liquid (ASL) and a defective antimicrobial capacity. Studies in CF patients are lacking to clearly understand the initial mechanisms that impair bacterial clearance.

Objectives: To investigate how the defect in CFTR function impacts on ASL pH and its relationship with antimicrobial capacity in human airways.

Methods: Real-time measurement of ASL pH from polarized cultures of human Cystic Fibrosis Bronchial Epithelial cell lines (CFBE 41o$^-$) cells and primary cells from healthy controls and F508del homozygous patients was investigated with a pH microelectrode in physiological (25 mM HCO_3^-, 5% CO_2) and extracellular normocapnic acidosis (10 mM HCO_3^-, 5% CO_2) conditions, upon pharmacological modulation of transporters of interest.

Bicarbonate secretion was assessed by short circuit current (SCC) in Ussing chamber upon mucosal-to-serosal HCO_3^- gradient across the epithelium in low Cl^- solutions.

Antimicrobial capacity of the airway cell cultures was studied after *S. aureus* CIP 76.25 (Collection de l'Institut Pasteur) apical infection by counting survival bacteria Colony Forming Unit at different time points.

The activity of the cathelicidin-related peptide, LL-37 and the hBD1 defensin were checked at different pH conditions.

Results: In both CFBE cell lines and primary cells, ASL pH was significantly lower in CF than in Wild Type (WT) CFBE 41o$^-$ cells and bronchial primary cells. CF cell lines and primary cells demonstrated a defect in ASL pH adaptation upon extracellular normocapnic acidosis. This was consistent with a defect in cAMP dependent HCO_3^- secretion by the CF epithelium, as assessed by SCC experiments. The anion exchanger SLC26A4 (pendrin), the non-gastric H^+/K^+ adenosine triphosphate (ATP12A) and CFTR at the apical side at the apical side contribute to pH homeostasis in human airways.

CF ASL demonstrated a defect in antimicrobial capacity which was improved by normalization of ASL pH in CF airways and related to a pH dependent LL-37 and hBD1 activities. Pendrin inhibition in WT airways recapitulated CF airway defect as assessed by acidified ASL and increased bacterial proliferation.

Conclusion: ASL pH impacts on its bacterial clearance capacity and the activity of the antimicrobial peptides. Increasing ASL pH might be one of the factors to improve innate airway defense in newborns with CF. Pendrin activation could represent a major therapeutic target.

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S6.6 Using *Xenopus tropicalis* to develop a live *in vivo* model of mucociliary clearance

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The deterioration of lung function caused by susceptibility to infection and inflammation are clinical manifestations of cystic fibrosis (CF). Production of thick, sticky mucus that is unable to be moved by normal mucociliary clearance mechanisms creates an environment where pathogens can thrive and overwhelm the body's immune response. A clear molecular understanding of how beating cilia transports mucus and how each of the components of the mucociliary clearance response interact and can be controlled is still lacking. This is due, in part, to the inherent difficulties in studying mucociliary epithelia *in vivo*, in real time. Here we present a new model to study live mucociliary clearance mechanisms - the skin of the *Xenopus tropicalis* tadpole.

As the *X. tropicalis* embryo develops into a tadpole, specialized cells form in the skin to protect the animal from environmental challenges and to exchange gases and nutrients. These cells include motile multi-ciliated cells that beat coordinately and goblet cells that secrete mucin glycoproteins onto the skin surface to form mucus. The tadpole skin is a mucociliary surface with great similarities to the respiratory tract of the human upper airways. But, unlike mammalian research models, it benefits from being open and accessible to the environment. This is particularly advantageous for *in vivo* research including live imaging.

Having very recently identified the major gel-forming mucin, MucXS, which is secreted onto the tadpole skin [1], we have now begun to use CRISPR-Cas9 genome editing at the *mucXS* gene locus for insertion of the sequence coding for the fluorescent protein mEGFP. We successfully targeted *mucXS* to induce a double strand break in the DNA that we repaired using a homology template containing mEGFP. Using an immunofluorescence approach we have shown co-localization of mEGFP with a MucXS antibody in the secretory vesicles. We sequenced the gene to show in-frame integration of mEGFP and are currently raising animals to generate a transgenic line. At the same time, we have also targeted the protein Tau to insert a red fluorescent protein that localizes to the cilia. We are at the stage where an appropriate guide RNA has been identified and are currently constructing the homology template. The aim is to be able to use advanced live imaging approaches to image each of the components together and study how the mucins interact with the cilia. Using this approach, we hope to identify important contributors to mucociliary clearance, mimic disease states and ultimately to translate our findings to human disorders of mucociliary clearance such as in cystic fibrosis.

We have also looked at the expression profile of the cystic fibrosis transmembrane conductance regulator (CFTR) in *X. tropicalis* skin from embryo to adult and suggest the development of a CF frog model to complement mammalian models, specifically to look at the effects on mucin secretion of mimicking mutations in CFTR.

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24 March — 11:00–12:45

S07: Therapeutic Approaches

S7.1 CFF therapeutics discovery beyond F508del

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For almost two decades, drug discovery efforts mostly focused on the most common cystic fibrosis-causing mutation of the CFTR gene, F508del. Now, there are multiple therapeutics advancing through clinical development that promise to bring highly effective therapies to patients with at least one copy of the CFTR F508del allele (similar to ivacaftor benefit for G551D patients). Including patients that can be treated with ivacaftor monotherapy, about 95% of all people with CF are expected to significantly benefit from these next generation therapies. Unfortunately, this leaves ~5% of patients without effective disease-modifying therapies.

For people with nonsense mutations, the N1303K mutation, or other minimal function mutations, few therapeutic approaches have yielded promising in vitro data and none have completed early human trials. While the CF Foundation continues to support the development of next generation modulators for CFTR F508del, essentially all new discovery and development programs focus on therapeutic approaches that target the CFTR variants that will not be treatable with the next generation treatments currently in later stage clinical trials. In addition to small molecule discovery for nonsense or premature truncation codon (PTC) mutations, multiple mutation-agnostic therapies are being pursued, including CFTR mRNA delivery, gene replacement therapies via viral vector delivery, and multiple variations of CFTR gene repair by gene editing. In alignment with these discovery priorities, the research at the CF Foundation's CFFT Lab is also primarily concentrating on the discovery process for the CF-causing mutations with an unmet therapeutic.

To that end, the CFFT Lab has screened ~200,000 small molecules for their ability to promote readthrough of premature stop codons. Several molecules were identified with convincing activity in FRT cells expressing CFTR cDNA constructs. However, the efficacy observed in the model cell system has not translated into primary patient-derived cells. The model cells suffer from non-native promoters and UTRs, lack of mRNA splicing, etc. We concluded that the available cell lines for screening and downstream assessment of potential PTC readthrough modulators have severe limitations in their ability to model the biology of CFTR PTC mutations. At the same time patient-derived cells with rare mutations are available only in limited quantity, and typically the rare mutation is only in one CFTR allele whereas the other allele is F508del. Deconvolution of experimental data for effects on the rare mutations is complicated to impossible.

Therefore, a significant effort was put towards generating better cell models. Through CRISPR/cas9 facilitated gene-editing of 16HBE14o- cells we generated homozygous cell lines expressing CFTR F508del and rare CFTR variants like G542X, N1303K, or W1282X from the native gene locus. In parallel, a new sensitive ELISA assay was developed that specifically detects full length CFTR. This assay is currently being adapted to a 384-well assay, eventually the screening for readthrough modulators of CFTR PTC mutations with an essentially native expression system.

The presentation will give a high-level overview of CFTR-directed therapies in development and illustrate challenges in novel therapeutic development with examples from the ongoing research at the CFFT Lab.

S7.2 Recent advances in gene and base editing

Patrick T Harrison

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Gene editing has been used to successfully create and correct individual CF-causing mutations in a range of cell types to model disease and explore the use of this approach as a potential therapeutic strategy. To explore the possibility of CFTR up-regulation as a potential therapeutic strategy, Cas9 variants have been developed which can directly up-regulate the expression of *CFTR* on a transient basis. Use of Cas9/gRNA editing has also led to the identification of novel regulatory elements within CFTR, which if deleted in a targeted manner, can increase mRNA levels on a permanent basis.

We have also used the targeted deletion strategy to correct individual deep-intronic CF-causing mutations with high efficiency. We are also exploring the use of homology-independent strategies which use the same DNA repair pathway to increase the efficiency of integration of CFTR superexon donors to correct multiple CF-causing mutations.

Recent studies have shown that the use of high fidelity Cas9 protein/gRNA particles can reduce off-target global effects, and the observation that long non-coding RNAs (lncRNAs) play in the repair process offers the possibility of novel strategies to further reduce specific undesirable off-target effects.

The final topic for discussion is the development of Cas9-base editing enzymes which can convert C:G to T:A base-pairs in DNA, or convert A:T to G:C base-pairs. These new tools have the potential to correct a subset of CF-causing mutations without creating double-stranded breaks in DNA, potentially reducing off-target effects; in particular they may be useful for the correction of certain PTC mutations. The use of these base editing Cas9 variants as a tool to create isogenic cell lines with reduced off-target effects is also discussed.

S7.3 Development of a PI3Kγ-derived peptide as a standalone therapy to activate F508del-CFTR, limit lung inflammation and promote bronchorelaxation in Cystic Fibrosis

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Background and Rationale: The underlying cause of Cystic Fibrosis (CF) is a mutation in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR), a cyclic AMP (cAMP)-stimulated chloride channel. The consequent CFTR dysfunction primarily affects the respiratory system, where the reduced activity of the channel results in obstruction of small airways and, together with airway inflammation and infections, eventually leads to respiratory failure. A number of CFTR correctors and potentiators, improving membrane expression and gating of the channel respectively, have been developed, but their ability to rescue the basic defect of CF is still unsatisfactory.

Hypothesis and Objectives: The ultimate goal of this project is to validate a novel therapeutic tool for CF. We previously showed that phosphoinositide 3-kinase γ (PI3Kγ) acts as a scaffold protein, which negatively regulates cAMP by favoring the activation of key cAMP-degrading enzymes, phosphodiesterases 3 and 4 (PDE3 and PDE4). We recently developed a cell-permeable peptide targeting the kinase-independent function of PI3Kγ. Here, we hypothesize that this molecule could be therapeutically exploited to rescue the conductance of the most prevalent CFTR mutant, F508del-CFTR. Moreover, targeting PI3Kγ scaffold activity may enhance cAMP in airway smooth muscle and immune cells, leading to concomitant bronchodilation and anti-inflammatory effects.

Methods: The biological function of the PI3Kγ-derived peptide was assessed in clinically-relevant CF models, namely human primary airway epithelial monolayers and primary intestinal organoids from F508del patients, as well as in a mouse model of chronic lung inflammation (OVA-sensitized mice).

Results: We found that, in vitro, the peptide potentiates F508del-CFTR and is significantly more efficient than the gold-standard CFTR potentiator VX-770 both in bronchial monolayers and in intestinal organoids. Furthermore, the peptide improves F508del-CFTR conductance even after chronic Orkambi®, a condition wherein VX-770 is ineffective, without interfering with channel stability. On the contrary, the peptide *per se* ameliorates F508del-CFTR stability at the plasma membrane.

Moreover, intratracheal instillation of the peptide significantly elevates cAMP in the lungs, limits methacholine-induced airway hyper-responsiveness, and reduces neutrophilic lung inflammation in OVA mice.

Conclusions: The PI3Kγ-derived peptide is covered by patent number TO2014A00110 - WO/2016/103176 and received the Orphan Drug Designation by the European Medicinal Agency (EU/3/17/1859) in 2017. We therefore intend to develop the PI3Kγ peptide as a novel medicinal product that could be used either alone or in combination with gold-standard therapies in F508del patients.

Overall, the results of this study demonstrate that the peptide targeting PI3Kγ may be exploited therapeutically to provide concomitant (i) CFTR potentiation, (ii) bronchodilation, and (iii) anti-inflammatory effects, eventually maximizing patients' benefit.

This work was supported by grants from the Italian Cystic Fibrosis Research Foundation (FFC#25/2014 and FFC#23/2015 to E.H., FFC#6/2016 to A.G) and Cariplo Foundation (#2015-0880 to A.G.).

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S7.4 New correctors rescue F508del-CFTR activity at low nanomolar concentrations

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The deletion of phenylalanine at position 508 (F508del) of the CFTR protein is the most frequent mutation among patients with cystic fibrosis (CF). This mutation causes a severe defect in protein folding and stability, and also affects the gating behavior. The folding and stability defect can be treated with compounds known as correctors, whereas the gating defect can be overcome by compounds called potentiators (1, 2). Only one corrector has been approved for the treatment of CF patients bearing the F508del-CFTR mutation, i.e. lumacaftor (VX-809), but only in combination with the potentiator ivacaftor (VX-770). The therapeutic benefit of the combination (Orkambi®) is however limited. There is therefore the need of new, more effective correctors.

The screening of a collection of about 15,000 maximally diverse commercial compounds, using FRT and CFBE41o- cells stably expressing F508del-CFTR and the Halide-Sensitive Yellow Fluorescent Protein (HS-YFP) (3), yielded two hits, belonging to different chemical classes. Rounds of chemical modifications of the hits and functional evaluation in different assays provided information on the structure-activity relationship for each chemical class.

One of the two classes was investigated more extensively, and the medicinal chemistry work led to a number of compounds with high potency and efficacy in rescuing the activity of F508del-CFTR in bronchial epithelial cells from CF patients homozygous for the F508del mutation, as measured by short-circuit current recordings.

The best correctors showed potency in the low nanomolar range, retaining very good efficacy at the concentration of 10 nM or even lower. The data generated on the most promising correctors will be presented and discussed.

This work was supported by the Italian Foundation for Cystic Fibrosis (FFC) as part of the "Task Force for Cystic Fibrosis" project.

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S7.5 *In vitro* characterization of clinical stage novel corrector PTI-801 and potentiator PTI-808 in primary airway cell models

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A phenotypic high-throughput screen that utilized a PTI amplifier to enhance the signal-to-noise ratio was performed. This strategy enabled the detection of raw hit compounds with modest functional activity that were missed in a screen of the same small molecule library that did not employ amplifier. PTI-801 is an optimized derivative of a small molecule identified using this strategy. Using immunoblotting, PTI-801 is shown to increase the levels of the mature glycosylated form of F508del-CFTR protein in primary HBE cells derived from *F508del* homozygous donors, consistent with its mechanism of action being that of a corrector. PTI-801 also increases the F508del-CFTR-mediated chloride channel activity in primary HBE cells in Ussing chamber measurements.

The corrector activity of PTI-801 shows specificity for CFTR, substantially increasing the maturation of F508del-CFTR protein, while having no impact on the maturation of a mutant form (G268V) of the related P-glycoprotein (PgP). In addition, the effect of PTI-801 on F508del-CFTR activity is maintained for 12 hours after removal of the compound, indicating that the PTI-801 functional benefit is on, or results in, a stable species of functional CFTR protein.

Like other correctors, PTI-801 is complementary with the potentiator ivacaftor, enhancing the function of F508del-CFTR in HBE cells from *F508del* homozygous donors to similar levels as those observed in cells treated with lumacaftor and ivacaftor. Unlike other correctors, under conditions in which PTI-801 is co-administered with ivacaftor, PTI-801 activity is maintained, exhibiting an enhanced *in vitro* efficacy relative to cells in which ivacaftor and lumacaftor are co-administered. In triple combinations, PTI-801 more than doubles the activity of lumacaftor and ivacaftor. This ability of PTI-801 to further enhance F508del-CFTR activity above that achieved with lumacaftor is consistent with the corrector activity of PTI-801 working through a mechanism of action that is distinct from lumacaftor.

To complement the PTI-801 corrector and PTI-428 amplifier in development at PTI, we have also developed PTI-808, a small molecule potentiator designed to rescue mutant CFTR chloride channel activity. PTI-808 enhances CFTR channel activity across partially purified channels, recombinant cell lines, and primary HBE cells from CF donors. Patch-clamp on isolated cell membranes expressing wild-type CFTR demonstrates that PTI-808 increases the open probability of the channel. Cell lines expressing recombinant G551D-CFTR showed increases in chloride current in response to acute additions of PTI-808, also consistent with PTI-808 acting through a potentiator-like mechanism on CFTR. Importantly, PTI-808 exhibits potentiator activity in Ussing chamber assays in HBE cells derived from CF donors who have gating- and conductance-defective CFTR mutations on one allele. The potency of PTI-808 between cell lines and HBE cells and between acute and chronic incubations, is consistent, exhibiting an EC_{50} of 2nM-12nM.

PTI-808 increases chloride transport in HBE cells derived from patients with one or two F508del alleles. The co-administration of PTI-808 with PTI-801and PTI-428 results in CFTR activity of 106% of normal CFTR function in F508del homozygous HBE cells, and 63% of normal CFTR in HBE cells with one allele of F508del and one allele of a nonsense mutation.

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S7.6 Restoration of CFTR function by antisense oligonucleotide splicing modulation

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A significant proportion of disease-causing mutations in humans affects pre-mRNA splicing. In Cystic fibrosis (CF), 10-15% of the mutations affect the correct splicing of the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene. Disease severity in patients carrying these mutations is highly variable, correlated with the level of aberrantly spliced transcripts. Antisense oligonucleotides (ASOs) were found to be highly efficient in modulation of the splicing pattern in several genetic diseases. We aim to develop an antisense oligonucleotides (ASOs) based therapy to modulate the level of correctly spliced CFTR. As a model we first focus on a common CFTR splicing mutation, the 3849+10kb C-to-T, which leads to inclusion of an 84 bp cryptic exon between exons 22-23 in the mature mRNA. This cryptic exon contains an in-frame stop codon that leads to degradation of a significant fraction of the mRNA by the NMD pathway as well as to the production of prematurely truncated nonfunctional proteins. We designed 2´-O-methyl phosphorothioate-modified ASOs, targeted to prevent the recognition of enhancer splice motifs in the cryptic exon or to mask the junctions between this exon and its flanking sequences. Screening of these AOSs led to the identification of several ASOs that significantly decrease the level of aberrantly spliced CFTR mRNA and increase the level of correctly spliced CFTR mRNA. Importantly we demonstrate that ASOs modulating this splicing pattern can restore the CFTR channel function in primary respiratory epithelial cells. Our results indicate that ASOs targeted to mask splicing motifs in the CFTR gene can increase the correct splicing of CFTR leading to improvement of channel function.

24 March — 14:30–16:15

S08: Translational CF Research

S8.1 Modeling patient specific responses to CFTR modulator therapies- Challenges and opportunities

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Goal/Objective: Currently- there is a need for standardized tools for predicting who will be responsive to expensive CFTR modulatory drugs. Human bronchial epithelial cells (HBECs) derived *from CF lung transplants* have had great utility in drug testing in the past. However, the current goal is to predict individual drug responses and recommend the appropriate treatment before an individual will need a lung transplant --hence there is a need to develop and validate new tissue based models that enable the implementation of patient specific therapies. We need reproducible methods for *in-vitro* drug profiling to enable evidence-based decision making regarding therapy implementation in CF. Our overarching goal is to determine which tissue based assays, will predict clinical outcome to CFTR modulator compounds. Toward this goal, we will employ a unique resource of patient derived tissue, developed through the Program in Individualized Therapy for Cystic Fibrosis or **CFIT**, to optimize and validate assays of approved drugs (and emerging interventions) in multiple tissue models derived from the same patient.

Progress/ Preliminary Results: The **CFIT** Program is tasked with generating a comprehensive resource from 100 CF donors, containing matched primary tissue (fresh nasal tissue) and iPSCs acquired/ generated prior to the initiation of modulator treatment. iPSCs were generated and quality control documented by the *Centre for Commercialization of Regenerative Medicine* (Toronto, Canada). The CFIT Program also determines gene expression (RNAseq) for drug naïve, fresh tissue sample from each individual and with recent funding from Genome Canada, gene expression for derived nasal cultures from each individual will be also determined. Whole genome sequencing using long read technology is being conducted and will enable unprecedented insights into the genetic background for each donor. Comprehensive clinical outcome data, pre- and post ORKAMBI treatment has been documented for each F508del/F508del patient recruited to this program. To date- we have 58 donors to this program with iPSCs available to the CF research community- worldwide.

This resource has enabled analysis of the correlation between *in-vitro* (Ussing chamber studies of nasal cultures) and clinical outcome assays for F508del homozygous patients. We found that there is large variability amongst individuals in both in-vitro and clinical responses to ORKAMBI. Importantly- there is a significant correlation between the ORKAMBI mediated change in mutant CFTR channel function in nasal cultures differentiated at air/liquid interface and percent predicted FEV1 (measured six months after treatment start date) for each individual ($p<0.006$). These findings support the potential of this culture model for predicting patient-specific responses to CFTR modulators. This resource also led to the development of high-throughput assays of patient specific epithelial tissues differentiated from iPSCs- assays suitable for profiling a large number of novel modulators/interventions for each individual. These examples highlight the opportunities provided by the systematic generation of a bioresource of patient-derived cell cultures.

As we progress toward an era in personalized medicine in CF- employing different models of patient-specific tissues- there are many key questions that need to be addressed. Most importantly- do our tissue culture models fully recapitulate the properties of the native tissue derived from each patient. The genetic, cell-based and clinical resources generated through CFIT will allow CF researchers to interrogate this key question.

Supported by SickKids Foundation and Cystic Fibrosis Canada

S8.2 Translating molecular and cellular knowledge in treatments for Cystic Fibrosis

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Major clinical advances in treating CF symptoms (with mucolytics, antibiotics, etc) have significantly increased survival beyond the second decade. However, to further increase CF patients life expectancy, CF needs to be treated beyond its symptoms i.e., through treatments addressing the basic defect associated with each CFTR gene mutation. Examples of new drugs such as potentiator VX-770/ ivacaftor, or VX-809/lumacaftor corrector in combination with ivacaftor demonstrate that this is a sound approach that brings effective benefit to individuals with CF. However, there are still >50% people with CF without a labelled drug for their genotype. To address the >2,000 CFTR mutations so far reported, they have been grouped into 7 functional classes which are now evolving into theratypes so as to be tackled by a common drug/ therapeutic strategy [1].

Our goal is to contribute to expansion of treatments addressing the basic CF defect to all patients with a two-fold objective: 1) to develop novel therapeutic strategies; and 2) to pre-assess in an effective and expedite way how rare mutations respond to approved and new CFTR modulator drugs as these become available. Together these approaches constitute the way forward to bring treatments to more patients CF, namely to those with rare ("orphan") mutations.

Towards the first objective we have developed cell-based microscopy assays to address mutations for less addressed theratypes and applied them in high-throughput (HT) microscopy screens, so as to identify: *i)* modulators of mRNA nonsense-mediated decay (NMD) (class I); *ii)* agents with ability to read-through premature termination codons (PTCs) (class I); *iii)* regulators of alternative splicing (classes I and V); *iv)* modulators of non-CFTR channels that can compensate for ion unbalance in CF in by-passing therapies [2] (all classes, including class VII).

Regarding the second objective, as for rare mutations "classical" clinical trials are not possible due to low numbers of patients and their geographic dispersion, we use methods to pre-assess directly on patient's cells/tissues how each individual responds to CFTR modulator drugs. These methods include the forskolin-induced swelling (FIS) assay in intestinal organoids [3] and measurements of CFTR-mediated Cl^- secretion in polarized primary cultures of conditionally reprogrammed cultures (CRC) of nasal epithelial cells (HNE) [4]. Such pre-assessment may become a standard basis for a drug clinical use in a precision medicine approach.

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S8.3 Personalized medicine, Task Force update

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The last decade has witnessed an unprecedented step change in the way we view CF treatments: the first mutation-specific small molecule CFTR modulators, and a massive increased in the clinical trial pipeline. Whilst extremely encouraging for people with CF, their families and care teams, this progress serves to highlight deep discrepancies in access of patients to licensed drugs (based both on genotype and geography).

The ECFS has convened a Task Force around what it perceives as the three major challenges in speeding up access to new therapies: the efficient design of clinical trials in a congested space and in the context of evolving standards of care; preclinical testing for rare mutations for example with the use of organoids; and inequalities in access to both clinical trials and licensed medications.

This presentation will feed back from a recent workshop engaging investigators, patient organisations, pharma and regulatory agencies and will describe our thoughts on the next steps in this process.

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S8.4 Drug repurposing for cystic fibrosis subjects with rare *CFTR* mutations

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The first-generation of clinically available CFTR-correcting compounds, ivacaftor (KALYDECO®, VX-770) and ORKAMBI® have elicited significant clinical improvements for people suffering from cystic fibrosis (CF) but have only been available to CF subjects harbouring 34 out of the more than 2000 reported *CFTR* mutations. Clearly, a need for new CFTR-modulating therapies for CF subjects with other (rare) *CFTR* mutations exists.

We have started the RAINBOW project in which we will screen >1400 FDA-approved drug compounds (including ivacaftor, lumacaftor and ORKAMBI®) in intestinal organoids derived from a total of 150 Dutch CF patients carrying rare uncharacterized *CFTR* mutations (with a prevalence of < 0.5% in the Dutch CF population), which include missense, frameshift, insertion/deletion, nonsense, and splicing mutations.

First, cytotoxicity of the FDA-approved drug compounds was assessed in heterozygous F508del/S1251N and homozygous F508del organoid cultures by performing a live-dead assay, assessing the intestinal organoid morphology and by determining the organoid swelling capacity upon forskolin induction after compound treatment. Based on these read-outs, 31 compounds (2.1% of the total library) were excluded from the FDA-approved drug library as these were too toxic to the organoids.

Next, we performed a primary screen, so far in 85 organoid cultures, by using our high-content 384-well forskolin-induced swelling assay (HTS-FIS) and identified several compounds capable of (partially) restoring CFTR function. To determine the optimal dosage and incubation time(s) of these compounds, we performed a secondary screen in parallel for further validation of these hits by using the conventional FIS assay (96-well screening format) and our recently developed steady-state lumen area (SLA) assay.

Altogether, our results show the potential of identifying new CFTR modulating compounds for CF subjects with rare, uncharacterized *CFTR* mutations by repurposing existing FDA-approved drug compounds.

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S8.5 Effects of lumacaftor-ivacaftor therapy on CFTR function in Phe508del homozygous patients with Cystic Fibrosis

Simon Y. Graeber[1,2,3], Christian Dopfer[4,5], Lutz Naehrlich[6,7], Lena Gyulumyan[4], Heike Scheuermann[1], Stephanie Hirtz[1], Sabine Wege[1,8], Heimo Mairbäurl[1,9], Marie Dorda[4], Rebecca Hyde[4,5], Azadeh Bagheri-Hanson[6], Claudia Rueckes-Nilges[6,7], Sebastian Fischer[4,5], Marcus A. Mall[1,2,3], Burkhard Tümmler[4,5]

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The combination of the CFTR corrector lumacaftor with the potentiator ivacaftor has recently been approved for the treatment of patients with cystic fibrosis (CF) homozygous for the Phe508del CFTR mutation. The pivotal phase 3 trials examined clinical outcomes, but did not evaluate CFTR function in patients. Hence, we wanted to examine the effect of lumacaftor-ivacaftor on biomarkers of CFTR function in Phe508del homozygous CF patients aged 12 years and older.

This prospective observational study assessed clinical outcomes including FEV1 % predicted and BMI, and CFTR biomarkers including sweat chloride concentration, nasal potential difference (NPD) and intestinal current measurement (ICM) before and 8-16 weeks after initiation of lumacaftor-ivacaftor therapy.

A total of 53 patients were enrolled in the study and 52 patients had baseline and follow up measurements. After initiation of lumacaftor-ivacaftor, sweat chloride concentrations were reduced by 18 mmol/L. Further, NPD and ICM showed partial rescue of CFTR function in nasal and rectal epithelia to levels of 10% and 18% of normal, respectively. All patients improved in at least one CFTR biomarker, but no correlations were found between CFTR biomarker responses and clinical outcomes.

We could show that lumacaftor-ivacaftor results in partial rescue of Phe508del CFTR function to levels comparable to the lower range of CFTR activity found in patients with residual function mutations. Functional improvement was detected even in the absence of short-term improvement of FEV1 % predicted and BMI.

P34

S8.6 *SLC6A14* overexpression modifies ΔF508-CFTR function in human bronchial epithelial cells

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Background and Rationale: Cystic Fibrosis (CF) is a common autosomal recessive disease that leads to pulmonary complications. Mutations in the *CFTR* gene, such as the most common ΔF508 mutation, can result in processing defects and/or abnormal function in the encoded HCO_3^- and Cl^- ion channel. Thus, current CFTR modulator therapies aim to improve CFTR expression, function, or trafficking to the cell surface, yet even the most recent FDA approved ORKAMBI™ (VX-809 and VX-770) therapy has modest and variable improvements on patient lung function. Genome-wide association (GWA) studies have shown that patient heterogeneity in CF disease severity (independent of CFTR genotype) is associated with modifier genes and recently, it has been proposed that the genotype of a certain modifier (*SLC6A14*) also correlates with therapeutic efficacy of CFTR targeted modulators. *SLC6A14* has been identified as a modifier of the CF lung phenotype and meconium ileus. It is an apical transporter of L-Arginine in the respiratory and colonic epithelium and we hypothesize that its arginine uptake activity will modulate CFTR channel activity through the Arginine-Nitric Oxide (NO) pathway. We were prompted to study the effect of SLC6A14 over-expression in the CF human bronchial epithelial cell line CFBE41o- to gain a better understanding of how this modifier gene affects F508del protein expression, function and response to modulators.

Objectives:

To define the effect of SLC6A14 over-expression in the CF human bronchial epithelial cell line CFBE41o- on F508del protein expression, function and response to modulators.

Methods:

Our lentiviral delivery system was designed with an open reading frame containing the *SLC6A14* gene tagged with GFP on the C-terminal end. Following transduction, intracellular nitric oxide (NO) was measured using a fluorescence based dye (DAF-FM diacetate). The electrogenic transport activity of SLC6A14 and F508del-CFTR channel activity was measured using the fluorometric imaging plate reader (FLIPR) assay.

Results: *SLC6A14* expression was confirmed by measurement of mRNA expression and visualization of GFP signal in confocal microscopy. Functional expression of SLC6A14 was confirmed by measurement of arginine evoked depolarization and arginine mediated NO production in SLC6A14-GFP lentivirus transduced cells. Interestingly, we observed an augmentation of the VX-809 rescued forskolin and CFTRinh-172-modulated F508del-CFTR channel activity at both 27 and 37 degrees in the SLC6A14 transduced cells.

Conclusion: *SLC6A14* overexpression significantly enhanced regulation F508del-CFTR channel activity after its rescue by VX-809. This cellular model supports the hypothesis that SLC6A14 function is a positive regulator of surface localized F508del-CFTR.

FP1.1 IL-17A impairs host tolerance during airway chronic infection by *Pseudomonas aeruginosa*

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Resistance and tolerance mechanisms participate to the interplay between host and pathogens. IL-17-mediated response has been shown to be crucial for host resistance to respiratory infections, whereas its role in host tolerance during chronic airway colonization is still unclear. Here, we investigated whether IL-17-mediated response modulates mechanisms of host tolerance during airways chronic infection by P. aeruginosa. First, we found that IL-17A levels were sustained in mice at both early and advanced stages of P. aeruginosa chronic infection and confirmed these observations in human respiratory samples from cystic fibrosis patients infected by P. aeruginosa. Using IL-17a(-/-) or IL-17ra(-/-) mice, we found that the deficiency of IL-17A/IL-17RA axis was associated with: i) increased incidence of chronic infection and bacterial burden, indicating its role in the host resistance to P. aeruginosa; ii) reduced cytokine levels (KC), tissue innate immune cells and markers of tissue damage (pro-MMP-9, elastin degradation, TGF-β1), proving alteration of host tolerance. Blockade of IL-17A activity by a monoclonal antibody, started when chronic infection is established, did not alter host resistance but increased tolerance. In conclusion, this study identifies IL-17-mediated response as a negative regulator of host tolerance during P. aeruginosa chronic airway infection.

Full Reference of the Paper: Sci Rep. 2016 May 18;6:25937. doi: 10.1038/srep25937. PMID: 27189736

FP1.2 Quorum Sensing Down-Regulation Counteracts the Negative Impact of *Pseudomonas aeruginosa* on CFTR Channel Expression, Function and Rescue in Human Airway Epithelial Cells.

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The function of cystic fibrosis transmembrane conductance regulator (CFTR) channels is crucial in human airways. However unfortunately, chronic Pseudomonas aeruginosa infection has been shown to impair CFTR proteins in non-CF airway epithelial cells (AEC) and to alter the efficiency of new treatments with CFTR modulators designed to correct the basic CFTR default in AEC from cystic fibrosis (CF) patients carrying the F508del mutation. Our aim was first to compare the effect of laboratory strains, clinical isolates, engineered and natural mutants to determine the role of the LasR quorum sensing system in CFTR impairment, and second, to test the efficiency of a quorum sensing inhibitor to counteract the deleterious impact of P. aeruginosa both on wt-CFTR and on the rescue of F508del-CFTR by correctors. We first report that exoproducts from either the laboratory PAO1 strain or a clinical <<Early>> isolate (from an early stage of infection) altered CFTR expression, localization and function in AEC expressing wt-CFTR. Genetic inactivation of the quorum-sensing LasR in PAO1 (PAO1ΔlasR) or in a natural clinical mutant (<<Late>> CF-adapted clinical isolate) abolished wt-CFTR impairment. PAO1 exoproducts also dampened F508del-CFTR rescue by VRT-325 or Vx-809 correctors in CF cells, whereas PAO1ΔlasR had no impact. Importantly, treatment of *P. aeruginosa* cultures with a quorum sensing inhibitor (HDMF) prevented the negative effect of *P. aeruginosa* exoproducts on wt-CFTR and preserved CFTR rescue by correctors in CF AEC. These findings indicate that LasR-interfering strategies could be of benefits to counteract the deleterious effect of *P. aeruginosa* in infected patients.

Maillé É, Ruffin M, Adam D, Messaoud H, Lafayette SL, McKay G, Nguyen D, Brochiero E.

Down-Regulation Counteracts the Negative Impact of *Pseudomonas aeruginosa* on CFTR Channel Expression, Function and Rescue in Human Airway Epithelial Cells.

Front Cell Infect Microbiol. 2017 Nov 10;7:470. doi: 10.3389/fcimb.2017.00470. eCollection 2017.

FP1.3 Analysis of long-range interactions in primary human cells identifies cooperative CFTR regulatory elements

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A mechanism by which control DNA elements regulate transcription over large linear genomic distances is by achieving close physical proximity with genes, and looping of the intervening chromatin paths. Alterations of such regulatory 'chromatin looping' systems are likely to play a critical role in human genetic disease at large.

Here, we studied the spatial organization of a ≈790 kb locus encompassing the cystic fibrosis transmembrane conductance regulator (CFTR) gene. Dysregulation of CFTR is responsible for cystic fibrosis, which is the most common lethal genetic disorder in populations. CFTR is a relatively large gene of 189 kb with a rather complex tissue-specific and temporal expression profile.

We used chromatin conformation at the CFTR locus to identify new DNA sequences that regulate its transcription. By comparing 5C chromatin interaction maps of the CFTR locus in expressing and non-expressing human primary cells, we identified several new contact points between the CFTR promoter and its surroundings, in addition to regions featuring previously described regulatory elements. We demonstrate that two of these novel interacting regions cooperatively increase CFTR expression, and suggest that the new enhancer elements located on either side of the gene are brought together through chromatin looping via CTCF.

Full Reference of the Paper Nucleic Acids Res. 2016 Apr 7;44(6):2564-76. doi: 10.1093/nar/gkv1300.

FP1.4 Anti-cystic fibrosis properties of the CB subunit of crotoxin: correction of ΔF508-CFTR chloride channel dysfunction

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The cystic fibrosis transmembrane conductance regulator (CFTR) is a plasma membrane protein belonging to the ATP-Binding Cassette (ABC) transporter superfamily. CFTR functions as an ATP- and PKA-dependent chloride channel regulating chloride flux across apical membranes of epithelial cells. Mutations in CFTR are the cause of Cystic Fibrosis (CF). The most common mutation, a deletion of Phe508 (ΔF508-CFTR) in the first nucleotide binding domain NBD1 of CFTR, affects the maturation and gating of CFTR. The search for new high affinity ligands of CFTR acting as dual modulators (correctors/potentiators) presents a major challenge in the pharmacology of CF.

We have recently shown that a snake venom phospholipase A2 (PLA2), the CB subunit of crotoxin from Crotalus durissus terrificus, is a novel binding partner for wt-CFTR and ΔF508-CFTR1. CB has an important beneficial function on the mutated ΔF508 chloride channel by interacting with NBD1 of human ΔF508-CFTR. CB exhibits both potentiation of CFTR chloride channel current and correction of misfolded ΔF508-CFTR1 and thus overcomes the deleterious effects of the ΔF508 mutation on the chloride channel function (gating and maturation of CFTR). The potentiating effect of CB on CFTR activity was demonstrated using electrophysiological techniques in X. leavis oocytes and in CFTR-HeLa cells and ex vivo in mouse colon tissue. The correcting effect of CB was shown by functional rescue of CFTR activity after 24 hours CB treatment of ΔF508-CFTR and by the presence of fully glycosylated CFTR. Molecular docking allowed us to propose a model of the CB/ΔF508NBD1 complex involving the ABCβ and F1-like ATP-binding subdomains of ΔF508-NBD1. HDX-MS analysis confirmed stabilization in these regions (also showing allosteric stabilization in two other distal regions). CB thus acts as a positive allosteric modulator of CFTR. Finally, SPR competition studies showed that CB disrupts the ΔF508CFTR/cytokeratin 8 complex, allowing escape of ΔF508-CFTR from degradation. This study opens novel perspectives for the development of anti-CF drugs.

1 Faure G, et al., Rattlesnake Phospholipase A2 Increases CFTR-Chloride Channel Current and Corrects ΔF508CFTR Dysfunction: Impact in Cystic Fibrosis. J Mol Biol. 2016 Jul 17;428(14):2898-915.

Full Reference of the Paper

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Rattlesnake phospholipase A2 as a novel dual-acting modulator of ΔF508 Cystic Fibrosis Transmembrane Regulator dysfunction

J. Mol. Biol. 428, 2898-2915.

24 March — 18:00–19:00

Closing Keynote Lecture

Pharmacological modulation of ion transport in CF: CFTR and beyond

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Restoration of chloride and bicarbonate transport in epithelial cells is a major therapeutic goal in cystic fibrosis (CF). This result can be achieved by targeting the specific molecular defects caused by CF mutations with small molecules. In particular, the severe defect in folding and trafficking of CFTR protein caused by the F508del mutation needs to be treated with combination of small molecules having complementary mechanisms of action.

In the last three years, we have worked at the identification of novel correctors and potentiators for F508del and other CF mutations. By screening a carefully assembled library of structurally-diverse small molecules (ca. 15,000) with a functional assay, we have found a panel of compounds that improve the biogenesis and activity of F508del-CFTR protein. In particular, two classes of F508del correctors were identified with significant activity in primary bronchial epithelial cells from CF patients. After several rounds of optimization, analogues belonging to one of such corrector classes revealed the ability to rescue F508del-CFTR with subnanomolar potency. Studies are under way to identify corrector combinations for maximal rescue of F508del-CFTR and other mutations sensitive to these new compounds.

An alternative way to correct the CF basic defect is the pharmacological modulation of other ion channels and transporters. TMEM16A (ANO1) is a second type of chloride channel expressed in epithelial cells. In theory, stimulation of anion transport through TMEM16A could circumvent the CFTR lack of function. By using genetic and pharmacological tools, we are evaluating the role of TMEM16A in the homeostasis of airway surface liquid (ASL). In parallel, we plan to screen a small molecule library to find TMEM16A activators/potentiators.

As a second type of alternative target, we are considering ATP12A, which is a proton pump expressed in the apical membrane of airway epithelial cells and a contributor to acidification of ASL in CF. We are studying the factors controlling the expression of ATP12A in airway epithelial cells and evaluating the possibility to discover specific inhibitors that could help to normalize ASL properties.

Our work is supported by Fondazione per la Ricerca sulla Fibrosi Cistica (FFC), Cystic Fibrosis Foundation (CFF), and Telethon Foundation.

P1

What can we learn from structural models of CFTR?

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Until recently, the lack of high resolution structures of different conformational states of CFTR has largely impeded atomic-scale understanding of the structural, energetic and dynamics effects of CF-causing mutations as well as rational drug design of CFTR modulators. This situation has dramatically changed with the publication of the coordinates of Zebrafish and human CFTR, in a dephosphorylated, ATP-less, inward-facing closed conformation and those of Zebrafish CFTR in a phosphorylated, ATP-bound, outward facing, near-open conformation. These structures are important for several reasons. First they demonstrate that CFTR is amenable to structural studies, using cryo Electron Microscopy (cryo-EM). This finding has already initiated multiple efforts towards resolving additional conformations of the protein. Second, these structures highlight new structural features of CFTR which may prove relevant for drug discovery. At the same time, these structures largely validated previously developed homology models of CFTR. This may suggest that available models that correspond to other conformational states of the protein are likely to be correct. Third, the structures serve as a tool to validate and interpret decades of efforts focusing on biochemical and functional studies. Finally, the structures could be used to initiate structure-based drug design efforts and perhaps help in developing mutation-specific therapies.

Despite this impressive progress, multiple challenges still remain. (1) The available structures only provide a static snapshot of the highly flexible and allosteric CFTR. (2) It is still not known which of the multiple CFTR conformations is sensitive to drug binding. (3) It is still not known where on CFTR, drugs are likely to bind. All these questions could be at least partially answered by solving additional structures of CFTR either in the apo state or in the presence of known modulators. However, until multiple CFTR-ligand complexes become available, answers and / or test-able hypotheses could be provided by computational techniques.

With this in mind, we present in this work a series of molecular dynamics (MD) simulations initiated from the EM structures of CFTR for both the WT protein and some of its mutants. We demonstrate that an analysis of the resulting trajectories could provide information on the stability of the EM structures as well as the effect of mutations. We also demonstrate how MD trajectories could be used for suggesting potential binding sites for CFTR modulators, and how such modulators could be docked into these sites. We propose that the hypotheses put forth by the computational analysis could be experimentally tested, e.g., by monitoring the effect of strategically designed mutations on ligand binding and by testing the compliance of the suggested sites with Structure Activity Relationship (SAR) data.

P2

Metadynamics simulations to study the transition between open and closed forms of the CFTR channel

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Cryo-electron microscopy has recently furnished valuable informations about CFTR 3D structure in inactive states (*Zhang et al. 2016, 2017, Cell ; Liu et al. 2017, Cell*). Before the release of these structures, our group have used comparative modeling and molecular dynamics simulations to propose models of human CFTR in an active conformation (*Mornon*, Hoffmann* et al., 2015, CMLS*) representing open and closed forms of the wild-type channel. Both conformers provided significant insights about CFTR structural and functional characteristics and offered a description of the possible architecture of the anion channel. These models, widely supported by various experimental studies, are also in good agreement with the now published cryo-EM experimental structures.

Despite these crucial structural data, we still need to further understand the transition mechanisms between the different conformational states and to evaluate the impact of mutations on these processes. In order to address these issues, we have for the first time exploited metadynamics simulations as a tool to unveil new insights into the channel dynamics and thermodynamics. This approach aims at i) enhancing rare events and thus overcoming standard molecular dynamics limitations and ii) obtaining the free energy landscape connecting two distinct states of the channel. A critical ingredient of the method is the choice of suitable degrees of freedom (referred to as collective variables) characterizing the transformation between the two states.

As a first step, we explored the path linking our open and closed models. Our simulation supplies a more accurate description of the transition between both states. The computation of the free energies associated with the conformers showed that the closed form is energetically favored over the open form with a free energy difference comparable to that provided by the hydrolysis of ATP. Furthermore, this simulation allowed us to observe correlated motions within NBD1, involving F508 and the sub-domain in contact with ATP, supporting their importance in CFTR functions.

As a second step, we also plan to combine current experimental and theoretical structural data for implementing our metadynamics setup, in order to characterize CFTR conformational transitions that are taking place between inactive (experimental) and active (our models) states. Thereafter, these structural data can be exploited to characterize the impact of CFTR mutations. Such information can subsequently be useful to i) highlight possible modulators binding sites, ii) characterize their interaction modes with known active molecules in an attempt to improve their efficiency and iii) rationalize the search for new specific compounds.

This work is supported by the Association Vaincre La Mucoviscidose (Paris, France).

P3

Biophysical and structural studies of purified CFTR and the effects of the F508del and G551D mutations

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The stability of CFTR is of crucial for disease progression and the F508del mutation has been shown to decrease the stability of the channel at physiological temperatures. Here we have examined purified CFTR in various detergents and with different phosphorylation states of the protein, as well as with known CFTR potentiator or corrector drugs (ivacaftor, lumacaftor, respectively). Using a fluorescent assay we show that purified F508del CFTR shows a lower stability at physiological temperatures and a lower thermal unfolding transition. These effects on the thermal stability appear to be fundamental to the specific amino acid change -- i.e. the overall 3D structure of the F508del and WT CFTR proteins appear to be very similar at low temperatures (287K) as judged by the extent of low-temperature labeling by the fluorescent compound as well as by preliminary cryo-EM studies. We have previously shown that the potentiator ivacaftor has a mild destabilizing effect on purified CFTR, whilst the corrector has a moderate stabilizing effect on F508del CFTR (Meng et al., 2017 doi: 10.1074/jbc.M116.751537). Here we present a wide survey of the effects of these drugs on different CFTR preparations and with different mutations. We examine not only the cooperative global thermal stability properties of these proteins, but also the extent of initial labeling of the fluorescent compound at low temperature which should detect any local unfolding or localized distortions of the native structure. There are consistent small effects detected in the thermal stability as well as the initial labeling with the fluorescent compound which indicates effects of the drugs on the native CFTR 3D structure and implies that they can directly bind to the purified protein.

P4

Nanobodies as tools to stabilize different conformational states of CFTR

Maud Sigoillot[1], Magdalena Grodecka[1], Marie Overtus[1], Daniel Scholl[1], Abel Garcia-Pino[2], Lihua He[3], Els Pardon[4], Toon Laeremans[4], Jan Steyaert[4], John Riordan[3], Cedric Govaerts[1]

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In most cases, Cystic Fibrosis is due to the deletion of the residue phenylalanine 508 (F508) in the first nucleotide binding domain (NBD1) of CFTR that prevents folding of the whole protein leading to its clearance by the quality control system and defects in channel gating. It is well established that F508 mutation affects NBD1 thermodynamic stability and possibly the interface between the NBD and the trans-membrane (TM) domain. Thus, chaperones that target directly NBD1 are the most likely to recover protein stability, a first step towards recovering transport activity. To understand the mechanistic basis of CFTR stabilization, we developed nanobodies as tools to lock different conformations of CFTR. We identified different families of nanobodies that bind to distinct epitopes of NBD1 of CFTR and stabilize the protein in different conformational states. Using X-ray crystallography, we solved the structures of the complexes and identified the epitopes at the atomic level. Our approach leads to the identification of high affinity CFTR-specific stabilizers, which opens new avenues to decipher CFTR folding mechanism and develop CFTR correctors. In addition, our high-resolution structural data surprisingly demonstrates that CFTR must be able to adopt conformations that depart significantly from the published cryo-EM structures, further establishing that CFTR is a highly dynamic protein, even under normal physiological regime.

P5

The conformational landscape of the regulatory insertion in NBD1

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Background: Amounting evidence indicates that the particular conformational dynamics of CFTR is linked to the regulation of its channel activity, but also to pathogenesis of Cystic Fibrosis. Specific structural features of CFTR have been identified as key elements involved in its conformational profile. A remarkable example is the regulatory insertion (RI), a 32-residue long segment in the first nucleotide binding domain (NBD1) of CFTR that has no counterpart in any other protein of the ABC transporter family. The RI is predicted to be intrinsically disordered and extremely mobile. While its function remains unknown studies have shown that deletion of the RI preserves channel function and enhances maturation. Furthermore, deletion of the RI thermally stabilizes CFTR allowing the channel to overcome the deleterious effects of the CF causing mutation ΔF508 also located in NBD1. Removal of the RI also allows cell-surface expression of the ΔF508 mutant.

Why is a destabilizing and apparently disordered segment conserved in all CFTR orthologs? Can we make use of the link between the RI and NBD1 stability to recover the latter?

Objectives: We aim to identify the conformational landscape of the RI to understand its native function and its relation to NBD1 dynamics, stability and the link to F508. Specifically we want to confirm whether or not the RI is unstructured or if it adopts specific conformations that are associated with different functions.

Methods: We use double electron-electron resonance (DEER) and single molecule FRET (smFRET) to monitor the dynamics of NBD1. On a cysteine-less NBD1 construct we engineered cysteine pairs that we can label with paramagnetic or fluorescent labels in order to monitor their relative movements. Furthermore we can modulate the conformational dynamics of NBD1 using our collection of NBD1-specific nanobodies.

Results: DEER measurements between cysteine pairs, with one residue inside the RI show the existence of several distances, indicating that some residues of the RI adopt discrete conformations. smFRET data confirms these trends and highlights the kinetics associated with the movements of the RI between conformations. Combining DEER and smFRET, we demonstrate that the dynamics of the RI can be regulated by binding of nanobodies to epitopes located on various parts of NBD1, highlighting the existence of a complex allosteric network in the protein.

P6

Nanobodies as conformational sensors of the rescue of ΔF508-CFTR

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Cystic fibrosis, more specifically the deletion of the phenylalanine 508 (ΔF508) of NBD1, leads to misfolding of CFTR and prevents its translocation to the plasma membrane. Therefore a large effort to develop molecules that aim to rescue the ΔF508-CFTR has been observed over the last years, but it remains unclear whether the native state of CFTR is recovered in response to those drugs. We aim to assess the conformational state of CFTR with nanobodies, the single variable domains of heavy chain only antibodies found in camelids. We isolated nanobodies that recognize different conformational epitopes of NBD1 and can thus be used as folding reporters of CFTR. We show, in BHK21, that the binding of the nanobodies to ΔF508-CFTR is modulated by correcting treatment such as lowering the temperature and VX-809. Another remarkable property of these nanobodies is their ability to stabilize NBD1. We are investigating the potential of nanobodies themselves to rescue the ΔF508-CFTR in living cells. In order to deliver the nanobodies inside the cells several strategies are followed. Purified nanobodies can be delivered using a cell penetrating peptide (such as TAT) or by osmocytosis and the use of a specific detergent (i.e. the iTOP method). The cellular tracking of the nanobodies is followed thanks to their fusion with a fluorescent marker. Alternatively, nanobodies are provided using mammalian expression vectors transfected in the cellular system if choice. Expression is monitored using microscopy and flow cytometry, demonstrating proper expression of GFP-fused nanobodies. In parallel we monitor the trafficking of CFTR to the plasma membrane by using cells stably expressing CFTR bearing an extracellular epitope. With the development of those tools we can now investigate the therapeutic potential of the nanobodies in living cells.

P7

CFTR folding and function, from the endoplasmic reticulum to the cell surface

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The ABC-transporter CFTR is a chloride channel that, when mutated, causes cystic fibrosis. It consists of two transmembrane spanning domains (TMDs), two nucleotide-binding domains (NBDs), and a unique regulatory region (R-region). Once at the plasma membrane (PM), phosphorylation and ATP binding and hydrolysis regulate CFTR gating. Though CFTR activity at the PM has been extensively investigated, little is known about its behavior in the endoplasmic reticulum (ER) and in the Golgi complex. Is CFTR active as a channel only when it reaches the PM? When and where, along the secretory pathway, does it acquire the structure that allows function? In order to answer these questions, our first aim is to identify the biochemical and structural differences that occur between the open and closed states of CFTR. We used metabolic labelling, to follow CFTR from the ER to the PM, combined with a protease-susceptibility assay to probe for conformational states of CFTR in living cells. Using HEK293T cells transiently expressing either WT, closed or open CFTR mutants, we found structural changes in the NBD2 domain and differences in the NBD1 domain of open CFTR compared to both WT and closed CFTR. Uncovering when and where CFTR acquires its functional structure in the secretory pathway, and the possible regulation(s) of this process, will help identify new targets of intervention for cystic fibrosis. Moreover, knowledge about the CFTR open and closed structures will provide us with valuable tools and information for testing mode of action of drugs and for future drug screening.

P8

Structure-guided allosteric corrector combination for ΔF508 and rare cystic fibrosis folding mutants

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The most common cystic fibrosis mutation, ΔF508 in nucleotide binding domain 1 (NBD1), impairs CFTR-coupled domain folding, plasma membrane (PM) expression, function, and stability. Robust ΔF508-CFTR correction is achieved by stabilization of NBD1, the interfaces between NBD1 and membrane-spanning domains (MSDs), as well as NBD2, the former two representing primary conformational defects, established by using combination of genetic and pharmacological means [1]. Thus, a rationally designed, structure-guided corrector strategy may require the combination of class I correctors supporting the NBD1-MSD1 and NBD1-MSD2 interface formation, class II correctors targeting NBD2, and class III correctors stabilizing the NBD1 domain [1].

VX-809 (lumacaftor), the only approved corrector, exhibits a class I mechanism and in combination with the gating potentiator VX-770 (ivacaftor) provides only modest clinical benefit to patients carrying two copies of the ΔF508 mutation [2].

Here we report the identification of compounds for all three corrector classes in a screen of ~600,000 small molecules by monitoring the PM expression of the HRP-tagged ΔF508 in CFBE41o- (CFBE) epithelia. Compounds that increased ΔF508 PM densities both in the presence and absence of VX-809 contained class III correctors, compounds that required VX-809 included class II correctors, and compounds exhibiting redundancy with VX-809 encompassed class I correctors. The mechanisms of action (MOAs) of correctors were determined by domain-interrogation and domain-specific binding assays, competition with reference compounds, and ΔNBD2-CFTR PM density measurements. While class I-III correctors alone displayed only modest correction, combination of correctors from all three classes synergistically increased the Δ508 PM density and function in CFBE by up to ~9 fold in comparison to VX-809 alone, augmented the mutant ER maturation and the abundance of the complex-glycosylated form, promoted the peripheral stability, and largely normalized the single channel function of ΔF508. These results correlated well with CFTR gain-of-function in human bronchial epithelia and human nasal epithelia from $CFTR^{\Delta F508/\Delta F508}$ patients. The effect of corrector combinations was determined on forskolin plus genistein activated and Inh_{172}-sensitive short circuit current and reached ~50% of the corresponding WT currents. Remarkably, corrector combination also rescued the functional PM expression defect of rare missense mutations, presumably by targeting common allosteric structural defects.

This study provides proof of principle for synergy screening to identify correctors with distinct MOAs a mechanistic framework for broad application of corrector combinations, which if used in structure-guided combinations can achieve therapeutically relevant correction levels of ΔF508 and other processing mutants.

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P9

Escape from the ER: Mechanisms for Correction of CFTR Misfolding

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Cystic Fibrosis (CF) is a fatal disorder associated with defective hydration of lung airways due to misfolding and premature degradation of the mutant Cl- channel F508del-CFTR. CFTR contains two membrane-spanning domains (MSD), two nucleotide-binding domains (NBD) and a regulatory domain. The formation of an active CFTR channel therefore requires: 1) proper subdomain folding, and 2) efficient subdomain assembly. F508del, which occurs in NBD1, reduces the thermodynamic stability of NBD1 and also hinders early and late biogenic steps required for CFTR assembly. Folding of F508del-CFTR therefore arrests at an intermediate state. Kinetically trapped F508del-CFTR folding intermediates are recognized by cytosolic Hsp70 and targeted for ERAD via the RMA1 and CHIP E3 ubiquitin ligase complexes. A subset of CFTR II mutants misfold and accumulate in a conformation that is recognized by Hsp70, but is difficult to degrade by ERAD. ERAD-resistant forms of misfolded CFTR enter complexes that contain ERAD machinery, but become stuck, so they must be cleared from the ER via a recently discovered ERQC-Autophagy pathway. Class I and Class II folding modulators act synergistically to suppress folding defects in CFTR and thereby accelerate the rate of CFTR folding, which enables F508del-CFTR biogenic intermediates to escape detection by ERQC machinery and traffic to the plasma membrane.

How misfolded CFTR is recognized by ERQC machinery is not clear. Likewise, the mechanisms by which Class I and II folding modulators alter the conformation of CFTR to promote its escape from the ER are largely unknown. We are addressing these questions to define the rate limiting steps in the folding of CFTR that must be overcome to increase the cell surface expression of F508del-CFTR and rare CFTR II mutants. Approaches taken to address these questions include cell based folding assays, structural modeling, and molecular dynamic simulations of CFTR assembly. Class I modulators were found to stabilize an N-terminal domain of CFTR that contains MSD1. Class I modulator action is dependent upon a network of amino-acids located at the interface between cytosolic surfaces ICL1 and NBD1. Class I modulators appear to stabilize the ICL1:NBD1 interface, which in turn helps stabilize F508del-NBD1. Class II folding modulators have little impact on the biochemical stability of MSD1, and instead promote the assembly of full-length CFTR into a conformation that is no longer recognized by the RMA1 E3 ligase. RMA1 interacts with DNAJB12 and Hsp70 to detect folding defects that appear to occur when F508del prevents the proper association of MSD1/NBD1 with MDS2. Models will be presented to describe how Class I and Class II modulators synergize to promote the folding/assembly of F508del-CFTR to a conformation can pass ERQC and traffic to the cell surface. This work was supported by grants from the NIH and North American CF Foundation.

P10

Role of PDZ domain binding in CFTR aggregation at the cell surface

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The cystic fibrosis transmembrane conductance regulator (CFTR) interacts with many proteins such as the scaffold protein sodium-hydrogen exchanger regulatory factor 1 (NHERF1). NHERF1 binding to a PDZ domain binding motif at the C-terminus of CFTR facilitates the recycling of CFTR to the plasma membrane from endosomes. Interaction with PDZ domain proteins also inhibits CFTR lateral mobility at the plasma membrane, reducing its apparent diffusion coefficient by ~5-fold in studies using image correlation spectroscopy (ICS) and fluorescence recovery after photobleaching (FRAP), and almost immobilizing quantum dot-labeled CFTR in single particle tracking experiments.

Recently we have also shown there are at least two populations of CFTR molecules on the plasma membrane of primary human bronchial epithelial cells (pHBE) under control conditions. Some channels are diffusely distributed while others produce punctate fluorescence due to their aggregation into clusters that are too small to be resolved by conventional optical microscopy. k-space ICS also revealed the presence of two dynamical populations, one displaying small spatial scale transport dynamics and confinement and another having larger spatial scale dynamics due to movement both outside and within microdomains. Dependence of the confined population on cholesterol suggested that it is in nanoscale lipid rafts. Finally, exposure to cell stressors such as viruses and cigarette smoke increased CFTR recruitment into clusters and their fusion into large ceramide-rich platforms. In this study we examined whether interactions with PDZ domain proteins such as NHERF1 are required for this CFTR clustering and platform formation.

To test this, a CFTR mutant lacking the C terminus (CFTR-ΔDTRL) was prepared in an adenoviral vector for comparison with adenoviral wt-CFTR. NHERF1 co-immunoprecipitated efficiently with exogenously expressed wt-CFTR but was not detected in CFTR-ΔDTRL pulldowns. When pHBEs were transduced with GFP-tagged CFTR-ΔDTRL, its distribution on the plasma membrane after several days was indistinguishable from that of wt-CFTR. No differences were observed in cluster density, degree of aggregation, or dynamical behavior. Under stress-inducing conditions, wt-CFTR and CFTR-ΔDTRL both formed large platforms and had quantitatively similar aggregation and dynamics. These results indicate that PDZ domain-CFTR interactions play little role in CFTR clustering and platform formation under these conditions, unlike maneuvers that alter membrane cholesterol or ceramide, which strongly affected both wt-CFTR and CFTR-ΔDTRL. Ongoing studies of pHBE cells that overexpress both NHERF1 and CFTR should reveal if endogenous NHERF1 levels are too low to regulate heterologous CFTR. Regardless, it is already clear that interaction with PDZ domain proteins is not required for CFTR aggregation in rafts and ceramide-rich platforms.

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P11

Therapeutic potential of HspB5 in cystic fibrosis

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Background: Many studies have shown that chaperones are implicated in the synthesis and maturation of WT- and mutated CFTR, of which Hsp40, Hsc70 and Hsp90 have been the most studied. We focused on HspB5 to evaluate its role and effect in cystic fibrosis (CF). HspB5, also called αB-crystallin, was identified in 1894 as a structural component of the eye and was recognized in 1991 as a member of the small Heat Shock Proteins (sHSPs) family. HspB5 is a ubiquitous protein which is mainly expressed in the heart, skeletal muscle, kidney, spleen, thymus, prostate, brain, crystalline and lung, therefore in tissues with a high level of oxidative metabolism.

Moreover, HspB5 presents interesting beneficial activities for the cystic fibrosis treatment: a chaperon-like activity that could improve the stability, maturation and transport to the apical plasma membrane of F508del-CFTR protein and an antioxidant activity that could correct the imbalance of the redox homeostasis induced by F508del-CFTR expression.

Aims: To study the therapeutic potential of HspB5 in CF.

Methods: We used control and CF mice lungs to assess HspB5 expression by western blot and immunohistology. We also used primary human nasal epithelial cells (HNEC), human bronchial epithelial cells (CFBE41o-) that stably express WT- or F508del-CFTR, HeLa and HEK cells. Cell surface expression of F508del-CFTR after HspB5 expression was assessed by three different technics: IF by confocal microscopy, ELISA and CFTR measurement at the membrane on living cells (Rotin's Lab, Toronto, Canada). Cell viability after oxidative stress was done.

Results: Endogenous HspB5 expression was increased in cystic fibrosis mice model lungs compared to control mice. In HNEC, the level of endogenous HspB5 protein changed depending on the number of days after the beginning of culture. Immunoprecipitation in HeLa cells showed that HspB5 can interact with WT- and F508del-CFTR proteins. We determined that the phosphorylation status of HspB5 was altered in F508del-CFTR-CFBE41o- in comparison to WT-CFTR-CFBE41o-. As HspB5 functions can be regulated by its phosphorylation, WT-HspB5 and phosphomimetic-HspB5 mutants (mutants that mimicked the presence or absence of phosphorylation) were used to test their ability to restore F508del-CFTR at the plasma membrane. We established that WT-HspB5 was able to partly correct the F508del-CFTR localization and that the phosphorylation status of HspB5 was essential to regulate this phenomenon. Finally, we showed that WT-HspB5 increased the survival after oxidative stress exposure of both WT-CFTR- and F508del-CFTR-CFBE41o- cells.

Conclusion: Our results support our hypothesis that HspB5 is a relevant target for the treatment of cystic fibrosis. The recent development of peptide aptamers and chemical molecules that modulate the sHsps intracellular activity and the clinical ongoing trials testing the safe *in vivo* delivery of recombinant HspB5 for multiple sclerosis treatment and chronic obstructive pulmonary disease, allow us to consider the rapid development of *in vivo* tests in cystic fibrosis context.

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P12

The autophagy inhibitor Spautin-1 antagonizes rescue of mutant CFTR through an autophagy-independent and USP13-mediated mechanism

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Background: The trafficking defect of CFTR protein caused by F508del mutation can be targeted with small molecules termed correctors. However, available correctors, such as VX-809 (Lumacaftor), do not fully rescue mutant CFTR. New therapeutic targets, involved in CFTR processing, need to be identified.

Aims: It has been shown that the ubiquitin specific peptidase 10 (USP10) is an important factor that controls CFTR degradation. Interestingly, a small molecule inhibitor of USP10 has been recently described. This molecule, called spautin-1, also inhibits another ubiquitin peptidase, USP13. By inhibiting USP10 and USP13, spautin-1 is also an inhibitor of autophagy, a process that has a possible important relationship with CFTR. Therefore, we are interested in evaluating spautin-1 as a possible pharmacological tool to perturb CFTR processing.

Methods: We modulated the expression of selected USPs by gene silencing/gene transfection and determined the effect on F508del-CFTR function.

Results: We found that spautin-1 antagonizes the rescue by VX-809 causing a rapid rundown of F508del-CFTR at the functional and molecular level. This effect may involve USP13 inhibition but is independent from autophagy block.

Conclusions: USP13 appears as an important protein regulating the fate of mutant CFTR while spautin-1 may become an interesting probe for mechanistic studies and the search of new therapeutic agents.

P14

Cis variants identified in F508del complex alleles modulate CFTR channel rescue by small molecules

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Molecules correcting the trafficking (correctors) and gating defects (potentiators) of the cystic fibrosis causing mutation c.1521_1523delCTT (p.Phe508del) begin to be a useful treatment for CF patients bearing p.Phe508del. This mutation has been identified in different genetic contexts, alone or in combination with variants in *cis*. Until now, 21 exonic variants in *cis* of p.Phe508del have been identified, albeit at a low frequency. The aim of this study was to evaluate their impact on the efficacy of CFTR directed corrector/potentiator therapy (Orkambi).

The analysis by minigene showed that two out of fifteen *cis* variants tested increased exon skipping (c.609C>T and c.2770G>A). Four *cis* variants were studied functionally in the absence of p.Phe508del, one of which was found to be deleterious for protein maturation c.1399C>T (p.Leu467Phe). In the presence of p.Phe508del, this variant was the only to prevent the response to Orkambi treatment.

This study showed that some patients carrying p.Phe508del complex alleles are predicted to poorly respond to corrector/potentiator treatments. Our results underline the importance to validate treatment efficacy in the context of complex alleles.

P15

Characterization of the rare Cystic Fibrosis-causing R560S mutation and its response to modulators

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More than 2,000 alterations have been described to date in the *CFTR* gene, most presumed to be pathologic. This wide spectrum of *CFTR* mutations comprises a large number of uncommon variants for which prediction of the disease outcome is difficult, since the respective functional defect is unclear. Many may result in partial (residual) CFTR function and milder "atypical" forms of CF but phenotypes may vary widely, thus posing considerable challenges in establishing a diagnosis and prognosis of CF. Elucidation of the molecular and cellular effects of mutations is likely to be a rich source of information about the structure and function of CFTR, and also useful to predict disease severity. Furthermore, this can also provide the scientific basis for development of targeted compounds for mutation-specific correction [1].

Our aim here was to characterize the rare R560S mutation using both patient-derived materials (rectal biopsies from one CF individual who is homozygous for this mutation) and also cellular models as well as to assess its responsiveness to CFTR modulators. R560S is located in NBD1 and was originally reported in the CFTR Mutation Database as causing a severe phenotype associated with pancreatic insufficiency.

Intestinal organoids were prepared from rectal biopsies and analysed by reverse transcriptase-PCR (to assess CFTR mRNA), by western blot (to assess CFTR protein) and by forskolin-induced swelling (FIS) assay [2]. A novel cell line expressing R560S-CFTR was also produced by stably transducing the CFBE parental cell line. Efficacy of CFTR modulators was assessed in both organoids and the R560S-CFBE cell line using VX-809, VX-661 and cysteamine (alone or in combination with epigallocathecin gallate, EGCG) and for the functional studies also potentiators (genistein and VX-770/Ivacaftor).

Our results show that

1) R560S does not affect CFTR mRNA splicing;

2) R560S affects CFTR protein processing, totally abrogating the production of its mature form;

3) R560S-CFTR evidences no function as a Cl^- channel;

4) None of the modulators tested rescued R560S-CFTR processing or function.

Altogether, these results indicate that R560S is a class II mutation. However, unlike F508del, R560S is not be rescued by any of the CFTR modulators tested here -- a finding previously reported for other class II mutations such as N1303K [3, 4] or G85E.

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P16

Study of W361R-CFTR mutation, a mild class 2 CFTR gene mutation

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Cystic fibrosis (CF) is caused by mutations of the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR). Mutations of the CFTR gene affect the trafficking of the protein and/or its function. Understanding the functional consequence of rare Cystic Fibrosis (CF) mutation is mandatory to adapt personalized therapeutic approach. Here, we studied the maturation and function of W361R-CFTR, a very rare CFTR variant. The mutation is a rare variant of CFTR found in European population and people carrying W361R in one allele are usually diagnosed lately, indicating a mild disease getting worse in time.

We used western blot, iodide efflux and patch clamp technics to investigate CFTR maturation, ion transport and pharmacology in BHK and HEK cells expressing pEGFP-CFTR-W361R mutant and -F508del for comparison.

As for F508del-CFTR, we failed to detect a mature fully glycosylated C-band form for W361R in western blot analysis. CFTR pharmacological correctors VX809 and VX661 (10µM 24h) and low temperature treatment (27°C 24h) are able to restore a detectable C-band in western blot. Interestingly we observed a significant iodide efflux for W361R-CFTR without correction treatment but not for F508del-CFTR. Whole cell patch clamp recording confirmed the functionality of the W361R mutant. Without correction, W361R-CFTR elicit a time and voltage independent current in presence of 10 µM Fsk + 30 µM Gst and are totally blocked by 10 µM of $CFTR_{inh}$-172 (current densities at +40 mV are respectively +21.6 ± 6.3 pA/pF and +1.7 ±0.7 pA/pF). Moreover, incubation with VX809 greatly increased the functional response of W361R (at +40 mV I=+89.9 ± 20 pA/pF) showing a good correction of the defective maturation. In addition, the comparison with F508del current density recorded after VX809 incubation (at +40 mV: +35.5 ± 5.6 pA/pF) showed a much better chloride activity for the W361R mutant suggesting an absence of functional defect compared to F508del-CFTR.

We identified W361R as a novel class 2 CF mutation with an abnormal maturation. However, a significant CFTR chloride activity was recorded without any correction and was magnified by CFTR correctors suggesting a "mild" class 2 mutation. Further experiment are needed to explain the functionality of the W361R mutant but we can hypothesis the presence of a small amount of the mature fully glycosylated form at the plasma membrane which was not detected by our western blot analysis.

Cryo-EM 3D structures of the closed apo-free and phosphorylated ATP-bound CFTR as well as models of the open conformer show that W361 lies at proximity of several class II mutations (E56K, P67L, L69H, R74W, G85E, P205S, L206W). Investigation of these class 2 mutations could be very useful for a better comprehension in CFTR folding.

P17

R334W CFTR, a severely compromised chloride conductance mutant, retains its bicarbonate conductance

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Bicarbonate transport function is impaired in CF particularly in the exocrine pancreas. Patients with severe CF become pancreatic insufficient early in life. Airway cells also secrete bicarbonate and unchecked acid secretion in the CF airways impairs the ability of the lung to defend itself against infection. Bicarbonate transport particularly in the exocrine pancreas occurs through a combination of CFTR and associated bicarbonate transporters. CFTR does conduct bicarbonate but to a much lower extent compared to chloride. To understand bicarbonate transport through CFTR we selected the R334W mutation with occurs in the 6^{th} transmembrane spanning segment of TMD1. Consistent with a reduction of the CFTR-dependent chloride transport which is rather insensitive to Ivacaftor, patients bearing the R334W mutation in combination with a second severe allele have high sweat chloride. Surprisingly, however, many are pancreatic sufficient. To study this further we created a CFBE41o$^-$ cell line stably expressing R334W using Flip-In technology. Western blot experiments detect mature band C consistent with some processing to the mature form. The steady state levels of mature band C are increased following treatment with either the proteasome inhibitor, MG-132, the aggresome inhibitor tubacin, the lysosomal inhibitor, E64 or the corrector combination C4+C18. To study the transport function of R334W compared to WT CFTR we cultured the cells on permeable supports and measured the short-circuit current. Currents were measured using a basolateral to apical chloride gradient, stimulated with forskolin and genistein and inhibited with CFTR inhibitor 172. We found that the CFTR-dependent Isc generated by R344W was approximately 1% that of WT CFTR. Currents could be increased by approximately 3 fold following treatment with the corrector combination C4+C18. To assess bicarbonate currents, we used a solution containing 125mM $NaHCO_3$ bubbled with 25% CO_2. Isc was measured with equal solutions in both chambers. In these experimental conditions the CFTR generated bicarbonate currents of R344W were nearly identical to those of WT CFTR. They increase above that of wt-CFTR when treated with the combination of correctors C4+C18. Given that bicarbonate was the major anion in the solution, the data suggest that R334W supports bicarbonate transport which is almost equal to that of WT CFTR. Taken together our data suggest that the chloride transport through R334W is severely compromised but bicarbonate transport is more like that of WT CFTR. This may explain why these patients have milder pancreatic function. Finally, patients with this mutation could be good candidates for combined corrector therapies. Funded by CFF.

P18

Lumacaftor-rescued F508del-CFTR has a reduced bicarbonate permeability

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Deletion of phenylalanine at position 508, F508del, the most frequent mutation among Cystic fibrosis (CF) patients, destabilizes the CFTR (cystic fibrosis transmembrane conductance regulator), thus causing both a folding and a trafficking defect. It leads to a dramatic reduction of CFTR expression at the epithelial cell surface. In cell models, several small molecules, called correctors, increase CFTR defective expression at the cell membrane, while small molecules called potentiators enhance the function of the CFTR channel. The enhancement of chloride transport of CF-human bronchial epithelial cells in vitro by the corrector lumacaftor (VX809) was confirmed in a clinical trial by the reduction of the abnormaly elevated sweat chloride values in a dose-dependent manner. However, there was not any improvement in the lung function of patients. With the aim to improve the therapy strategy, the combination of lumacaftor with the potentiator ivacaftor (Orkambi™) was further tested, but the results have been disappointing. The most common cause of morbidity in CF is lung disease, caused by an uncontrolled homoeostasis of the apical surface of the airways. In airway epithelium, fluid secretion is driven by the osmotic force generated by chloride transport, so that the failure of CFTR leads to a reduction of the airway surface liquid (ASL), arresting the ciliar clearance. But CFTR transports bicarbonate as well, thus, in CF subjects there is a significant reduction of the bicarbonate concentration, and a lower pH, in the ASL, compromising the mucins' post-secretory process, resulting in an increased mucus viscosity. Although lumacaftor seems able *in vitro* to target the CFTR to the plasma membrane and partially restore the chloride transport, we propose that the lack of positive clinical results is due to the impairment of the CFTR-mediated bicarbonate transport that is still compromised in the rescued F508del-CFTR. To test this hypothesis, we determined the relative chloride to bicarbonate permeability ratio, $P_{Cl/HCO3}$, of wild type (WT) and rescued F508del-CFTR in permanently transfected FRT cells using the patch clamp technique. As predicted, the lumacaftor-rescued F508del-CFTR shows a reduced relative bicarbonate permeability, with $P_{Cl/HCO3} > 9$, compared with the characteristic $P_{Cl/HCO3} \approx 3$ found in the WT-CFTR channel. These results, which are consistent with our previous structural data (Baroni et al, CMLS 72:1363, 2015), would indicate that the rescue of the F508del-CFTR with lumacaftor yields a folding which is unable to carry out all the expected functions of the protein. Interestingly, the rescue of F508del-CFTR by low temperature incubation (27°C) does not compromise the relative bicarbonate permeability, opening the possibility of a correct folding of the rescued-mutant CFTR.

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P19

Identification of variants in *cis*-regulatory sequences of the *CFTR* gene

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The *Cystic Fibrosis Transmembrane conductance Regulator* (*CFTR*) gene was identified in 1989. Although, more than 2000 mutations have been discovered, patients with cystic fibrosis or *CFTR*-related disorders (*CFTR*-RD) have incomplete genotypes or present extreme phenotypes. The regulatory elements described in the promoter, cannot alone explain the complex tissue-specific regulation of this gene. In recent years, the development of chromatin conformation study techniques has identified several long-range regulatory elements as involved in this control expression (Moisan et al., 2016). This project allow to study the involvement of '*cis*-ruption', that is the dysfunction of a *cis*-regulatory element, in cystic fibrosis and one of its *CFTR*-RD, Congenital Bilateral Absence of Vas Deferens (CBAVD), in patients with extreme phenotypes (early or late *Pseudomonas aeruginosas* colonization) or incomplete genotype. 17 *cis*-regulatory sequences of the *CFTR* gene were sequenced by a hight-throughput approach (Fluidigm) in about 100 patients. This study allowed the identification of variants, some of which are described in the GnomAD database. Several variants in our study group are significantly more found than in the general population ($p< 10^{-5}$). These more frequent variants could be associated with cases of cystic fibrosis or CBAVD. Moreover, several variants of these frequent are located in regulatory element binding sites, and therefore could be regulatory variants, involved in chromatin organization and *CFTR* gene expression.

P20

Integrated approach to assess CFTR gene expression in organoids

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Differences in gene expression may contribute to the marked phenotypic variation that is observed among CF patients with identical CFTR genotype. Such differences may also account for the variable response to drug (corrector/potentiator) therapy. Efforts to monitor gene expression have mostly focused on measuring mRNA levels (for example, using microarray or RNA-seq), although the mRNA levels do not mirror protein concentration. We use deep-sequencing based ribosome profiling (Ribo-Seq) to assess gene expression in cultured organoids (so called colonoids) grown from stem cells in rectal biopsies from homozygous F508del patients and healthy controls and examined at different stages of cellular differentiation. Ribo-seq provides information on translating ribosomes with nucleotide resolution and thus, is informative on the regulation of gene expression at the level of translation. Our aim is to unravel in ion channel transportation or gene expression during the development of intestinal epithelium.

P21

Development of an enhanced Cystic Fibrosis transmembrane regulator gene construct for homology-independent targeted integration in Cystic Fibrosis cell lines

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The comparatively easy non-invasive accessibility of the lungs through aerosols and the monogenic nature of the disease makes Cystic Fibrosis (CF) an attractive candidate for gene therapy and genetic editing. However, *in vivo*, the many technical barriers including: inefficient vectors for gene transfer, the mucus barrier and immune surveillance mechanism, results in a very low efficiency of gene/genetic editing system delivery. It is suggested that ~10% wild type (WT) cystic fibrosis transmembrane regulator (CFTR) expression within target cells *in vivo* is sufficient for prevention of lung disease. This level of gene transfer/correction in patients has not yet been achieved and it is a controversial estimate. CFTR is a multifunctional protein and 10% WT activity may not restore some essential functions sufficiently to prevent CF disease progression. However, cells over expressing CFTR have been shown to restore bicarbonate secretion and antimicrobial functions in monolayers more efficiently than endogenous expression, whilst pharmaceuticals that act to increase the open probability (Po) of mutated forms of CFTR have shown to increase the potential for anion secretion.

We have therefore constructed an enhanced CFTR gene ($CFTR_{enh}$) with codon optimisation and containing two mutations (K978C and K190C) which have been described to permit a >2 fold increase in CFTR Po. K978C and K190C Mutations were inserted into the construct sequentially by site directed mutagenesis and were then sequenced to ensure success. We have shown that this construct was successfully processed into CFTR protein that translocated to the membrane when transfected into human cell lines HEK 293T and H441. Preliminary data showed that, under activity of identical mCMV promoters, $CFTR_{enh}$ transfected H441s produced greater abundance of CFTR protein as observed via western blot than CFTR WT transfected H441s. Assays for functional activity are ongoing.

We have flanked the $CFTR_{enh}$ gene with guide RNA sites specific for Intron 1 of human CFTR and incorporated an EGFP marker for intended use in Homology-independent targeted integration (HITI). We plan to integrate the enhanced CFTR gene into semi immortalised CFBE BMI-1 cells, downstream of the endogenous CFTR promoter. Given that more protein was detectable from $CFTR_{enh}$ gene than that of WT CFTR gene, even at low transfection efficiencies, provides data to support that correcting CF cells with this form of CFTR may restore function with a lower (and achievable) rate of genetic correction.

P22

An αLISA-based approach for determining CFTR protein levels across different types of cells and samples

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We have identified a new class of CFTR modulators, known as "amplifiers", represented here as PTI-CH, that are known to modulate CFTR by specifically increasing CFTR mRNA and protein levels. Previously, we presented the development and validation of a novel CFTR ELISA (Bialek et al. ATS 2017 (San Francisco, CA, USA) and ECFS 2017 (Seville, Spain) which has been developed to monitor CFTR protein levels from cell lines, human bronchial epithelial cells (HBEs), and clinical samples (nasal brushings). Moreover, we showed that with this novel CFTR ELISA, PTI-CH treatment of WT-HBE and CF-HBE cells increased CFTR protein in a dose-dependent manner, correlating to the effects observed on mRNA by qPCR, as well as western blot analysis.

Recently, we have developed a novel AlphaLISA assay that can be used to monitor CFTR protein levels based on our in-house ELISA. The AlphaLISA technology has many advantages over traditional ELISA. It is even easier and faster to use, compatible with many types of samples, requires no washing steps, and offers a very high sensitivity and large dynamic range. AlphaLISA is a bead-based assay technology that can be used for screening in a microplate format. It requires labeling of two bead types: Donor (Streptavidin coated) and Acceptor. Donor beads contain a photosensitizer, phthalocyanine, which converts ambient O_2 to an excited and reactive form of O_2, upon illumination at 680 nm. If the Acceptor beads are within proximity of the excited O_2 electron, energy is transferred from the oxygen to the Acceptor bead and leads to light production at 615 nm. The signal generated is proportional to the amount of analyte present in the sample.

Two publicly available CFTR antibodies were used for AlphaLISA development: An UNC 596 antibody which recognizes the NBD2 domain (aa 1204-1211) and UNC 450 which recognizes the R domain (aa 696-705) of CFTR protein. We showed that biotinylation of either UNC 450 or UNC 596 led to similar signal to noise ratios. The assay has been validated with CFBE F508del cells treated with the amplifier PTI-CH compared to vehicle and the performance was comparable to our in-house ELISA. The assay is compatible with automation, and could provide a highly-sensitive quantification approach for screening for new CFTR modulators. This AlphaLISA CFTR protein methodology has the potential to be a useful tool for clinical studies as well.

P23
Label-free measurement of native CFTR

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Reporter-based assays of CFTR expression and maturation in engineered cell lines have supported important mechanistic work on channel maturation, the effect of disease-causing mutations thereon, and the discovery of modulators that correct F508del and other mutants. However, these approaches are currently of limited utility for studying nonsense mutations whose liabilities only fully manifest themselves when in the context of the native CFTR gene. Moreover, the availability of CFTR variants expressed in 16HBE, iPS, and primary cells obviated a need for measurement of native CFTR expression and maturation in cells that cannot be conveniently engineered for reporter assays. To address this need, we developed a CFTR-based enzyme-linked immunosorbent assay (ELISA) capable of monitoring CFTR in primary cells. Such a label-free measurement also eliminates concerns about potential effects of reporter modification on the behavior of CFTR.

The assay is a classic sandwich format using two CFTR specific antibodies for capture and detection. For utility in study of nonsense mutations our objective was to develop a method sensitive only to full-length CFTR protein. Thus, one antibody was against an extreme C-terminal epitope; and the other against NBD2. The assay was first implemented in a colorimetric 96-well format. Assessing multiple mammalian cell models (e.g. isogenic FRT and gene-edited 16HBE14o$^-$ cells expressing CFTR variants), we have demonstrated that the method detects full-length CFTR under conditions where the truncated product of CFTR W1282X is undetectable.

The assay was evaluated for three applications: HTS, hit validation, and drug theratyping. In a 384-well plate format, the assay allowed identification of compounds that promote readthrough of CFTR premature termination codon (PTC) variants and compares favorably to reporter-based methods. It is also useful for hit validation based on two hundred hits from the reporter-based HTS tested for CFTR restoration. Given the improved throughput of the ELISA versus classic Western blot, it provides an attractive intermediate assay for hit progression after primary screening. Another application of the ELISA is assessing the efficacy of the existing CFTR corrector (e.g. VX-809) on rarer genotypes. To that end, in cell lysates from differentiated hBE cells expressing F508del CFTR, VX-809-induced CFTR expression was clearly detected. Thus, the method has sufficient sensitivity for VX-809-based theratyping in primary cells.

The CFTR-based sandwich ELISA provides a sensitive, label-free approach for biochemical detection of CFTR, which may be applied to screening, hit validation, and theratyping. A particularly attractive feature of this direct assay is that it can be applied to relevant natively CFTR expressing cells.

P24

A robust and sensitive flow cytometry based assay to quantify the cell surface density of Cystic Fibrosis transmembrane conductance regulator mutants

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Introduction: Cell models are invaluable to characterize rare CFTR mutations. Human immortalized cell lines overexpressing CFTR variants can rapidly be generated and insertion of a tag allows easy CFTR detection. Human intestinal organoids provide a physiological model to study CFTR processing and can be grown in 3D or 2D. Several methods have been described to quantify CFTR at the plasma membrane (PM) using immunofluorescent, colorimetric or chemiluminescent read-outs. The above described assays are all performed on monolayer grown adherent cells and provide a global quantification of cell surface CFTR. Flow cytometry allows assessing CFTR cell surface density at the single cell level in a highly quantitative, sensitive, and reproducible manner.

Aims: We aimed to set up a flow cytometry assay in 96-well format to study trafficking and quantify PM density of three processing mutations (G85E, F508del, N1303K), located in distinct regions of CFTR. Methods: We introduced 3HA into the 4th extracellular loop of CFTR (WT, F508del, G85E, N1303K) for sensitive detection of PM CFTR and generated cell lines using lentiviral vectors (LV) in HEK293T, HeLa and A549. Organoids were transduced with the same LV. PM CFTR was visualized by immunocytochemistry (ICC) and quantified by flow cytometry. Western blotting (WB) was performed to assess the amount of (im)mature CFTR. The effect of corrector VX809 (2.5 µM, 24 h) on CFTR processing was studied.

Results: All cell lines revealed residual PM expression of N1303K on ICC and flow cytometry, but not for G85E. Whereas some residual PM expression for F508del was present in HeLa's, barely any was detectable in HEK293T or A549. Treatment with VX809 rescued F508del (~7-fold) and to some extent N1303K (~2-fold) but not G85E to the PM. WB revealed no mature CFTR for G85E, the hallmark of class II mutations, under basal conditions, whereas for N1303K a very weak, diffuse band C was detected. None of the mutants showed residual short circuit currents in 2D organoids transduced with LV, but WT-CFTR resulted in recovery of CFTR activity similar to endogenous CFTR. CFTR expression in organoids was verified by ICC and CFTR PM density by flow cytometry is currently ongoing.

Discussion: In contrast to traditional classification of processing mutations based on absence of band C, our studies in cell lines indicate that the three processing mutations studied show different defects based on PM density. Our results support the use of the flow cytometry trafficking assay as a complementary method for determining in an easy and reliable manner the cell surface density of CFTR variants. Combined with the continuously expanding portfolio of primary cell models, this work will contribute to a better understanding of the multiple defects caused by a large number of CFTR mutations and provide a solid basis for personalized medicine strategies in the future.

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P25

Identifying new therapeutic targets for CF airway disease using real-time dynamic measurements of the ASL pH

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Background: Targeting the acidic airway surface liquid (ASL) pH in CF could potentially restore bacterial killing, ASL hydration and mucociliary clearance. Although both CFTR and the non-gastric H^+/K^+-ATPase ATP12A have been implicated in pH regulation, other transporters and ion channels might also be involved. In order to identify new therapeutic targets for CF, we need to understand how ASL pH is regulated in non-CF cells under near *in vivo* conditions. Therefore, the aim of this study was to determine the contribution of ion channels and transporters, as well as signalling pathways in the regulation of ASL pH.

Methods: The ASL of polarised non-CF primary bronchial epithelial cells was stained with a mixture of pHrodo, a pH-sensitive fluorescent dye (0.1mg/ml) and the pH-insensitive AlexaFluor488 (0.1 mg/ml), diluted in a modified Krebs solution, overnight at 37°C, 5% CO_2. The next day, readings were performed every 5 min in a temperature and CO_2 equilibrated plate-reader. Agonists and inhibitors were added either with the dyes if their target was located apically or after 2hr of baseline readings if their target was on the basolateral membrane. The effect of agonists and inhibitors was also measured on short-circuit current (Isc) in Ussing chambers either in the absence of Cl^-, or under a basolateral to apical HCO_3^- gradient.

Results: A rise in [cAMP]i (using 10µM forskolin) alkalinised the ASL ($t_{1/2}$ = 35 min) which was blocked by the PKA inhibitor H89 and the CFTR inhibitor CFTRInh172. Surprisingly, CFTRInh172, in the absence of forskolin, did not affect resting ASL pH. This result was correlated to an absence of response in resting Isc to CFTRInh172 in Cl^--free or HCO_3^- gradient conditions. Carbonic anhydrase and apical Na^+-H^+-Exchanger were not involved in resting nor forskolin-induced ASL pH regulation but inhibiting the Na^+-Bicarbonate Cotransporter (NBC), using 30µM S0859, reduced resting and forskolin-induced increases in ASL pH. Interestingly, the inhibition of the calcium-activated chloride channel TMEM16A, by 10µM T16-A01, did not affect resting ASL pH, but further increased the response to forskolin. Finally, inhibiting ATP12A with apical ouabain (100µM), significantly increased ASL pH. However, ouabain treatment disrupted epithelial integrity as evidenced by a decreased TEER (130±17 $\Omega.cm^2$ versus DMSO: 451±110 $\Omega.cm^2$) and cells showed little response to agonists in Ussing chambers, suggesting that overnight treatment with ouabain was deleterious to primary airway epithelium.

Conclusion: Our technique enables stable ASL pH measurements to be obtained from primary airway epithelial cells under thin film conditions, and gives an insight into the kinetics of the responses to different agonists and inhibitors. An increase in [cAMP]i alkalinised the ASL in non-CF cells which was CFTR dependent. Importantly, we show for the first time the lack of involvement of CFTR in resting ASL pH regulation as well as the deleterious effect of chronic ATP12A inhibition on the epithelium, suggesting that ATP12A might not be an appropriate target to restore ASL pH in CF. However, our results identified ANO1 and NBC as potential new therapeutic targets.

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P26

Novel regulators of F508del-CFTR identified by means of a functional genomics approach in bronchial epithelial cells of human origin: some possible mechanism of action

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Background: The identification of proteostasis regulators important for the processing of mutant CFTR, is one possible approach to reducing CF's detrimental health. Using a high-throughput functional assay to screen an siRNA library, we identified 37 proteins whose silencing results in a significant rescue of F508del-CFTR activity. The identified targets include proteins associated to F508del-CFTR degradation (like UBA52 and UBXN6) and sumoylation (i.e. UBE2I, UBA2), transcription factors (i.e. MLLT6, CHD4, TRIM24) and also proteins with unknown function like FAU.

Aim: Now our work is focused on: 1. the characterization, prioritization and dissection of the mechanism(s) of action by which downregulation of selected proteins leads to mutant CFTR rescue; 2. the evaluation of global changes induced by target downregulation on cell morphology and functions.

Methods: The effects of silencing/overexpression of specific targets has been evaluated on rescue of mutant CFTR processing and concurrent possible pleiotropic effects by means of biochemical and electrophysiological techniques and by high-content imaging and analysis.

Results: While some of the targets appears particularly promising and specific for CFTR (like FAU), other can be associated to deleterious effects due to dysregulation of specific cell pathways and/or to undesired effects on ion transepithelial transport as in the case of TRIM24 suppression resulting in ENaC upregulation.

Conclusions: We have evidenced a panel of proteins that are important for the processing of mutant CFTR, paving the way for using these proteins, and possibly their pathways, as new therapeutic targets to rescue mutant CFTR function.

P28

Deficiency of Ca^{2+}-mediated Cl^- secretion is a pathogenic feature of chronic rhinosinusitis

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Background: Chronic rhinosinusitis (CRS) is one of the most common chronic disorders of the upper airways that is frequently observed in patients with cystic fibrosis (CF), a rare genetic disease caused by abnormal epithelial ion transport. CRS is a heterogeneous disease which may be linked to impaired mucociliary clearance and sinonasal inflammation. However, other underlying CRS pathophysiologic mechanisms still remain unspotted. Here, we aimed to study whether the epithelial ion transport, in particular the Ca^{2+}-mediated Cl^- secretion is affected in patients with CRS. We further determined the epithelial expression of cytokines being both, associated with ion transport and implicated in CRS.

Method: First, cultured nasal epithelial primary cells of healthy individuals and CRS patients were utilized to study epithelial ion transport and transcript levels of the Ca^{2+}-activated Cl^- channel TMEM16A, cytokines (IFN-γ, IL-13) and purinergic receptors (P_2Y_2, P_2Y_4). Acute treatment with the type 2 cytokine IL-13 was also investigated. Second, nasal potential difference (PD) measurements were carried out in healthy individuals and CRS patients.

Results: Bioelectric studies revealed significantly reduced basal I_{sc} and Ca^{2+}-activated Cl^- secretory responses ($p< 0.05$), albeit mRNA levels of the Ca^{2+}-activated Cl^- channel TMEM16A and inflammatory cytokines (IFN-γ and IL-13) inducing Cl^- secretion, were significantly increased in CRS cultures ($p< 0.05$). We next compared UTP-mediated I_{sc} in CRS and control cultures in the absence and presence of IL-13 pretreatment. By acute treatment with IL-13, Ca^{2+}-activated Cl^- secretion can be partially restored in CRS cultures and TMEM16A expression was further increased ($p< 0.05$). Transcript levels of the P_2Y_2, but not P_2Y_4, receptor triggering Ca^{2+}-activated Cl^- secretion were decreased in CRS cultures to 42.1±7.0% ($p< 0.05$) and may partially explain the functional deficiency. *In vivo* nasal PD measurements confirmed a significant reduction of Ca^{2+}-activated Cl^- secretion in CRS patients compared to healthy individuals ($p< 0.05$).

Conclusion: Our data suggest a complex dysregulation of ion transport dominated by reduced basal I_{sc} values and Ca^{2+}-activated Cl^- secretion as well as of the cytokine signature of nasal epithelial cell cultures of patients with CRS. Changes in Ca^{2+}-activated Cl^- secretion can also be found *in vivo.* These data indicate that TMEM16A-mediated Cl^- secretion may be implicated in the pathogenesis and TMEM16A may serve as a therapeutic target in CRS.

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P29

Reduced glucose-induced membrane potential oscillations in F508del mouse pancreatic β-cell: contribution of Anoctamin 1 (Ano1)

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In basal conditions, pancreatic beta cell membrane potential is quite stable (~-70 mV). However, increase in extracellular glucose concentration above some threshold value (5-7 mM) induces cyclic fluctuations of the membrane potential. The initial depolarization, due to closure of the K_{ATP} channels, is driven by increased ATP generated by increased glucose entry and metabolism; then alternating depolarization (active) and repolarization (silent) phases occur as a wave whose frequency depends on the extracellular glucose concentration. This wave pattern is at least partly explained by Ano1 gating (1) and is reminiscent of the slow wave observed in intestinal interstitial cells of Cajal also involving Ano1 gating. During the active phases, bursts of action potentials (AP) occur suggesting that the threshold for opening voltage-dependent Ca^{2+} channels is reached, allowing Ca^{2+} entry and triggering insulin release. We have studied the fluctuations of the beta cell membrane potential by patch clamp analysis in aged F508del mice (>1 year) and compared them to those occurring in matched WT mice. In F508del mice, the resting membrane potential was hyperpolarized (-81 vs -74 mV in WT; p=0.0002) and the number of AP/30 min was drastically reduced (811 vs 1394 in WT; p=0.01) with the second peak amplitude partially repolarized (-29 vs -15 mV in WT; p=0.009). Moreover, the silent phase between the two first peaks was increased (275 vs 715 sec in WT; p=0.025).

To further analyze the possible contribution of Ano1, we studied the effect of the Ano1 potentiator bromophenyltetrazolbenzamide (Fact) (2). When Fact was added simultaneously with the increased glucose concentration, no silent phase between the two first peaks was anymore observed and the number of AP/30 min was increased from 1394 to 3475 (p=0.0002). When Fact was added after the increase in glucose concentration, the AP events/30 min increased from 1728 to 3240 (p=0.01) while the AP peak was further depolarized (-16 vs -21 mV; p=0.007).

In preliminary experiments, we found that the Ano1 potentiator Fact has a similar effect in F508del as in WT beta cell, thus increasing AP number and partially correcting the abnormal electrical activity of F508del beta cells.

In conclusion, we showed that defective CFTR induces membrane hyperpolarization and a dysfunction in Ano1 gating with reduced number of AP/30 min. We also showed Fact appears to correct Ano1 dysfunction in F508del mice and could have a potential interest in CF-related diabetes.

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P30

ATP12A as an alternative therapeutic target in cystic fibrosis lung disease

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ATP12A is a possible therapeutic target in cystic fibrosis (CF). It is expressed in the apical membrane of the airway epithelium where it is responsible for proton secretion coupled to potassium absorption. In CF epithelia, ATP12A activity, combined with the defective bicarbonate secretion due to CFTR loss of function, causes abnormal acidification of airway surface liquid (ASL) that leads to impaired antimicrobial activity and increased mucus viscosity (Shah et al., Science 2016). The importance of ATP12A and, in general, of ASL pH in CF has been questioned by a recent paper reporting that airway surface is not acidic in children with CF (Schultz et al., Nat Commun 2017).

To evaluate the role of ATP12A in CF lung disease we studied its expression and function in human airways. Immunofluorescence experiments carried out on histological sections of freshly excised non-CF bronchi revealed a very low expression of ATP12A. Instead, in CF samples, ATP12A was consistently detected on the surface epithelium, particularly on the apical membrane of non-ciliated cells and in submucosal glands. We also investigated ATP12A expression in vitro, in cultured bronchial epithelia exposed to conditions mimicking CF airways, such as treatment with bacterial components or inflammatory cytokines. Different stimuli, such as bacterial supernatant from *Pseudomonas aeruginosa* and IL-4, strongly increased ATP12A expression in both CF and non-CF cultures, suggesting that infection and inflammation may be responsible for ATP12A upregulation in CF airways.

To evaluate ATP12A function and role in the regulation of ASL pH we used a microelectrode. A small volume (75 µl) of saline solution with low buffer capacity was added to the apical side of well-differentiated CF and non-CF bronchial epithelia; after three hours, the solution was recovered and pH measured. In CF epithelia the measured pH was significantly acidic (7.3 ± 0.06) compared to that measured in non-CF (7.67 ± 0.06; $p < 0.001$). Furthermore, by adding a solution containing a higher concentration of potassium, to facilitate ATP12A-dependent proton pumping, we observed a larger ASL acidification in CF vs non-CF epithelia (deltapH = 0.4 ± 0.12 and 0.08 ± 0.05 in CF and non-CF, respectively; $p < 0.01$). Importantly, addition of ouabain, a non specific ATP12A inhibitor, reduced acidification by 50%.

Our findings confirm the acidic ASL pH in CF epithelia and support the role of ATP12A as a therapeutic target in CF. Indeed, pharmacological inhibition of ATP12-mediated proton secretion could antagonize acidification in CF airways and help to normalize mucus properties and restore antimicrobial activity.

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P31

Measuring water transport, CFTR and LRRC8 activities in human airway epithelial cells: contribution of quantitative phase microscopy

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Background: In airway epithelia, the cystic fibrosis transmembrane conductance regulator (CFTR) plays an important role in mucus hydration. A dysfunctional CFTR can lead to the production of viscous mucus that traps bacteria, dust and parasites which can cause infections and inflammatory reactions. These pulmonary manifestations are the main cause of mortality among Cystic Fibrosis (CF) patients. Thus, the physiological regulation of water and ion transport in the respiratory tract is crucial. Water homeostasis depends on aquaporins (AQP) that can mainly transport water across membranes of biological cells and participate to cell swelling or shrinking. An other protein named VRAC for volume-regulated anion channel, an heteromer of LRRC8 isoforms, is activated in response to a rapid cell swelling to restore the cell volume. Recently, we showed that CFTR can activate the AQP3 in CHO cells and stimulate an efflux of water accompanying the efflux of Cl^- (Jourdain et al., 2014). Moreover, in CHO cells expressing F508del-CFTR, no activation of water transport were observed.

Objectives: Our goal is to study the interactions between CFTR, AQP and VRAC in CF and non CF human airway epithelial cells.

Methods: Identification of aquaporins and LRRC8 isoforms expressed in CHO, 16HBE and CFBE cells was performed by RT-PCR. The development of the Quantitative Phase Microscopy (QPM), a label-free technique, to measure water transport, allowed us to correlate variations in the optical path difference (OPD) with water flux following the activation or absence/inhibition of CFTR and VRAC. In parallel, the patch-clamp technique in whole-cell configuration was used to study CFTR and VRAC activities in CHO and human pulmonary epithelial cells.

Results: Our results showed that AQP3 and LRRC8a/e isoforms are expressed in CHO, 16HBE and CFBE cells. In CHO cells expressing wt-CFTR, we observed an increase of OPD in 38% of the cells after application of Forskolin (10 µM, 5 min) corresponding to water efflux. No variation of OPD was detected in CHO K1 cells that are lacking CFTR. These results obtained with the QPM are consistent with the conclusions made by Jourdain et al. (2014). Moreover, during the application of a hypoosmotic choc to CHO cells, we observed a decrease of OPD, corresponding to water influx.

Using the patch-clamp in whole-cell configuration, we activated a chloride current in response to the perfusion of a hypoosmotic solution (Δ70 mOsM). This current was abolished in presence of DCPIB (20 µM), a VRAC inhibitor, showing that it was dependent of the VRAC activity. Similar experiments on 16HBE and CFBE cells are in progress.

The development of QPM, a non invasive cellular method, to study water transport in epithelial cells could allow to test the effect of potential therapeutics on mucus hydration in CF.

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P32

Role of pendrin (SLC26A4) in secretion by primary bronchial epithelial cells

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Anion secretion by airway epithelial cells is essential for mucociliary clearance. Cl^- drives most fluid secretion while HCO_3^- secretion has several functions which include mucin unpacking and bacterial killing. Despite their importance in the pathogenesis of CF and other respiratory diseases, anion efflux mechanisms at the apical membrane of human airway epithelial cells remain uncertain. Pendrin is expressed in control airways and is elevated in murine models of chronic obstructive pulmonary disease and asthma. Upregulation of pendrin is also observed in vitro when primary epithelial cell cultures are treated with pro-inflammatory cytokines such as IL-4/IL-13 or IL-17A. The aim of this study was to examine the role of pendrin in anion secretion by primary human bronchial epithelial cells under control conditions and when exposed to IL-4.

Pendrin was constitutively expressed at low levels. IL-4 (10 ng ml^{-1}) treatment greatly increased (by ~112 fold) the amount of pendrin mRNA in human bronchial epithelial cells compared to untreated control cells (n=6; $p< 0.001$). Immunofluorescence staining confirmed the upregulation of pendrin by IL-4 at the protein level and localized it at the apical membrane. After pretreatment with IL-4, intracellular pH (pH_i) measurements during challenge with low Cl^- solution revealed a 4-fold increase in apical anion exchange (AE) activity in the absence of forskolin (n=8 from 2 donor). AE was not inhibited by DIDS or CFTRinh-172, however specific knockdown of pendrin using adenoviral siRNA abolished the anion exchange activity, providing further evidence that it is mediated by pendrin in IL-4 treated cells.

Interestingly, forskolin (10 mM) caused a further increase in apparent AE activity, which was also enhanced after IL-4 pretreatment and diminished in pendrin knockdown cells. pH_i and Ussing chamber measurements revealed that elevating pendrin expression had at least two distinct effects, it directly increased pendrin-mediated anion exchange and also enhanced CFTR activation by cAMP. The latter is reminiscent of regulatory protein-protein interactions between CFTR and other SLC26A transporters that have been reported previously. To test this possibility more directly, we measured whole cell anion current in BHK cells stably expressing CFTR alone or together with EGFP-pendrin. Forskolin-stimulated whole cell currents mediated by CFTR were 50% larger in cells that co-expressed pendrin (n=7-8; $p< 0.001$).

We conclude that pendrin mediates significant anion exchange and also enhances electrogenic anion flux through cAMP-activated CFTR channels when its expression in primary human bronchial epithelial cells is upregulated by IL-4.

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P33

Early neonatal mortality of *Slc26a9* deficient mice is triggered by airway mucus obstruction

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Background: The epithelial Cl^- channel SLC26A9 (member of the SoLute Carrier family) has been associated with a higher susceptibility to muco-obstructive lung diseases like bronchiectasis and asthma (Bakouh N. *et al*., 2013 and Anagnostopoulou P. *et al*., 2012). SLC26A9 has been further identified as a modulator of lung function in Cystic Fibrosis (CF) patients treated with CFTR-directed therapies (Strug LJ. *et al*., 2016), supporting the role of SLC26A9 as a modifier of CF and potentially other muco-obstructive lung diseases.

Aim: The aim of this project was to determine the role of SLC26A9 during early postnatal adaptation *in vivo*.

Methods: We compared the pulmonary phenotype of wild-type (WT) and *Slc26a9* deficient (*Slc26a9*$^{-/-}$) mice on a C57BL/6 background including survival, histology, µCT imaging analysis and oxygen saturation measurements. On a functional level, transepithelial potential differences (PD) of cultured tracheal explants of WT and *Slc26a9*$^{-/-}$ mice were assessed.

Results: At birth, all genotypes were represented according to Mendelian genetics. In the first 30 minutes of life *Slc26a9*$^{-/-}$ mice presented irregular breathing resulting in a decreased survival of 48% compared to WT mice ($p< 0.05$). To determine the cause of death, histological analyses were performed and revealed mucus plugging in the trachea, proximal and distal airways in deceased *Slc26a9*$^{-/-}$ mice compared to WT mice ($p< 0.01$ for all levels analyzed). Preliminary results from µCT imaging studies support early onset airway mucus obstruction associated with atelectasis in *Slc26a9*$^{-/-}$ mice. By measuring the effect of mucus plugging on ventilation *Slc26a9*$^{-/-}$ mice showed significantly reduced oxygen saturation of 32% compared to WT mice (80%, $p< 0.01$). To evaluate the contribution of SLC26A9 Cl^- channel to the epithelial ion transport in the neonatal lung, PD was measured from cultured tracheas. Basal PD was lower in *Slc26a9*$^{-/-}$ (-18 ± 6 mV) compared to WT mice (-34 ± 6 mV; $p< 0.05$).

Conclusions: Taken together, the early phenotype is dominated by severe mortality due to mucus obstruction being associated with decreased oxygen saturation and decreased epithelial ion transport in vivo. Our data support that SLC26A9- mediated Cl^- secretion plays a critical role in airway mucus clearance in the neonatal lung and that this process is essential for normal postnatal adaptation.

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SLC6A14 overexpression modifies ΔF508-CFTR function in human bronchial epithelial cells

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Background and Rationale: Cystic Fibrosis (CF) is a common autosomal recessive disease that leads to pulmonary complications. Mutations in the *CFTR* gene, such as the most common ΔF508 mutation, can result in processing defects and/or abnormal function in the encoded HCO_3^- and Cl^- ion channel. Thus, current CFTR modulator therapies aim to improve CFTR expression, function, or trafficking to the cell surface, yet even the most recent FDA approved ORKAMBI™ (VX-809 and VX-770) therapy has modest and variable improvements on patient lung function. Genome-wide association (GWA) studies have shown that patient heterogeneity in CF disease severity (independent of CFTR genotype) is associated with modifier genes and recently, it has been proposed that the genotype of a certain modifier (*SLC6A14*) also correlates with therapeutic efficacy of CFTR targeted modulators. *SLC6A14* has been identified as a modifier of the CF lung phenotype and meconium ileus. It is an apical transporter of L-Arginine in the respiratory and colonic epithelium and we hypothesize that its arginine uptake activity will modulate CFTR channel activity through the Arginine-Nitric Oxide (NO) pathway. We were prompted to study the effect of SLC6A14 over-expression in the CF human bronchial epithelial cell line CFBE41o- to gain a better understanding of how this modifier gene affects F508del protein expression, function and response to modulators.

Objectives:

To define the effect of SLC6A14 over-expression in the CF human bronchial epithelial cell line CFBE41o- on F508del protein expression, function and response to modulators.

Methods:

Our lentiviral delivery system was designed with an open reading frame containing the *SLC6A14* gene tagged with GFP on the C-terminal end. Following transduction, intracellular nitric oxide (NO) was measured using a fluorescence based dye (DAF-FM diacetate). The electrogenic transport activity of SLC6A14 and F508del-CFTR channel activity was measured using the fluorometric imaging plate reader (FLIPR) assay.

Results: *SLC6A14* expression was confirmed by measurement of mRNA expression and visualization of GFP signal in confocal microscopy. Functional expression of SLC6A14 was confirmed by measurement of arginine evoked depolarization and arginine mediated NO production in SLC6A14-GFP lentivirus transduced cells. Interestingly, we observed an augmentation of the VX-809 rescued forskolin and CFTRinh-172-modulated F508del-CFTR channel activity at both 27 and 37 degrees in the SLC6A14 transduced cells.

Conclusion: *SLC6A14* overexpression significantly enhanced regulation F508del-CFTR channel activity after its rescue by VX-809. This cellular model supports the hypothesis that SLC6A14 function is a positive regulator of surface localized F508del-CFTR.

P35

The inhibition of calcium-activated potassium channel KCa3.1 increase ASL in acute isolated mouse tracheas

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Introduction and aims: The respiratory tract is lined by a thin layer of fluid, the airway surface liquid (ASL), which plays an important role in the mucociliary clearance (MCC). ASL is composed by a mucus layer that contains secreted mucins and the periciliar fluid layer that keeps the mucus separated from the epithelial surface. ASL volume is critically regulated by sodium absorption and chloride secretion in order to maintain the MCC function. Measurement of ASL height on primary airway cell cultures by confocal microscopy is a powerful tool which has allowed the study ASL physiology and pharmacology, nevertheless, the use of mouse cells requires an elevated number of animals per culture. We have successfully adapted the use of a fluorescent probe to determine ASL height in the acute isolated mouse trachea. We asked whether changes in fluid absorption relate to ASL height using the *Kca3.1*$^{-/-}$ mice that showed a significant reduction of ENaC mediated sodium absorption.

Methods: To measure the ASL thickness, freshly isolated mouse tracheas were placed in a humidified chamber bathed in physiological buffer (37° C) under constant flow and ASL was labelled with 8 µl PBS containing 2 mg ml^{-1} Texas red--dextran (10 kD). Tracheas were then placed in a sealed chamber and images were obtained in XZ-scanning mode using a Olympus FV1000 confocal microscope with a water immersion lens (20×/1.0 numerical aperture) and exited at 540/440 nm. We imaged the ASL height at 10-20 min after dye application and up to 150 min. Images were processed using the ImageJ software and Texas Red-dependent fluorescence intensity along the *z*-axis was calculated. After derivation of the intensity signal, curves with Gaussian functions were fitted to identify the points with higher rate of fluorescence increase or decrease to determine the distance between these two points. The distance obtained corresponds to the thickness of the ASL. Finally, the mean of the values obtained from the entire scanned area, corresponds to the ASL height.

Results: The ASL height of wild type mice and *Kca3.1*$^{-/-}$ was similar at the starting time (40 µm). However, 60 minutes after addition of the dye the mean ASL height was higher in *Kca3.1*$^{-/-}$ trachea (18.43 ± 1.003 µm; n=4) than wild type (11.41 ± 0.4730 µm; n=9) and the difference was maintained up to 150 min.

Conclusions: We successfully determined ASL height in acute isolated mouse tracheas of mice. Our results demonstrate that the reduced sodium absorption by KCa3.1 inhibition causes an increase in the ASL height which could explain the enhanced MCC previously observed. Preliminary results using tracheas from the Beta-ENaC transgenic animal indicate a reduction in ASL height as predicted for the augmented absorption of sodium. We are now adapting the technique to test the effect of agonists and inhibitors of ion channels of interests for the maintenance of ASL and MCC.

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P36

PMCA pump dysfunction causes Ca^{2+} overload and pancreatic ductal cell damage in cystic fibrosis

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Introduction: The cystic fibrosis transmembrane conductance regulator (CFTR) has a major role in pancreatic ductal secretion and its genetic defects damage the pancreas. It is known that intracellular Ca^{2+} homeostasis is disturbed in bronchial epithelial cells in cystic fibrosis (CF), but the connection of CFTR and the intracellular Ca^{2+} signaling has never been suggested in pancreatic damage in CF before.

Aims: Our aim was to characterize the Ca^{2+} homeostasis of CFTR-deficient PDEC.

Materials and methods: Wild type (WT) and CFTR knockout (KO) mouse pancreatic ductal (PDEC) and acinar cells (PAC), human CF pancreatic cell line (CFPAC-1) and human pancreatic organoids generated from induced pluripotent stem cells (IPSC) of controls and CF patients were used in the study. Intracellular Ca^{2+} levels, mitochondrial membrane potential ($\Delta\Psi_m$) and mitochondrial morphology was assessed using fluorescent probes and transmission electron microscopy, respectively. Immunofluorescent staining and quantitative PCR measurements were performed to detect changes of protein expressions. Protein ligation assay (PLA) was performed to detect contact between proteins.

Results: The plateau phase of the agonist-induced Ca^{2+} signal was significantly elevated in CFTR KO PDEC caused by decreased function of the plasma membrane Ca^{2+}pump (PMCA). Functional inhibition of CFTR had no effect on the PMCA activity. Whereas native CFPAC-1 cells, CF human organoids and murine PDEC treated with siCFTR showed similarly impaired PMCA activity. On the other hand, different strategies to restore the CFTR expression, such as Sendai virus mediated gene delivery in CFPAC-1, or VX-809 treatment of CF organoids completely restored PMCA function. As a downstream consequence, sustained $[Ca^{2+}]_i$ elevation decreased $\Delta\Psi_m$ and released cytochrome c in CFTR KO PDEC without significant alteration of mitochondrial morphology. Immunostaining revealed the colocalisation of PMCA4 and CFTR on the apical membrane of polarized, primary PDEC and PLA confirmed the intimate proximity of the proteins. Calmodulin, a possible link among CFTR and other proteins, showed plasma membrane localization in WT PDEC, which was shifted to the cytosol of CFTR KO cells.

Conclusion: Impaired expression of the CFTR leads to disturbed Ca^{2+} homeostasis and mitochondrial damage in primary PDEC due to the decreased activity of PMCA. These changes can contribute to the pancreatic damage seen in cystic fibrosis.

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Bicarbonate secretion is dependent of NBCe1 in airway epithelial cells of the mouse trachea

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Introduction; Bicarbonate transport is important for maintaining airway surface liquid (ASL) homeostasis. Bicarbonate transport reduction acidify ASL and, therefore, can alter mucus viscoelasticity, negatively affecting mucuciliary clearance function in the lungs. The aim of our study was to describe bicarbonate transport in acute isolated mouse tracheas and evaluate if the NBCe1 co-transporter plays a role in airway bicarbonate transport.

Methods: We performed Ussing chamber experiments in freshly isolated mouse tracheas (21 and 60 days old animals) to determine short circuit currents. We used acutely isolated airway epithelium to evaluate changes in pH using BCECF. Immunolocalization of NBCe1 in mouse airways tissues was performed by immunofluorescence.

Results: UTP-evoked anion current increased with age (-52.5 ± 9 vs -118 ± 11 $\mu A*cm^{-2}$, at days 21 and 60 respectively). When a bicarbonate-free bath solution was used anion current was significantly reduced (-29.8 ± 3 and -41 ± 4 $\mu A*cm^{-2}$, at days 21 and 60 respectively), a similar effect was observed when the inhibitor of NBCe1 S0859 was used in bicarbonate buffer (-34 ± 9 and 57 ± 8 $\mu A*cm^{-2}$, at days 21 and 60 respectively). This observation was correlated with UTP-induced acidification in acutely isolated airway epithelium, an effect that was significantly reduced when S0859 was added. We found that NBCe1 expressed in both Clara and ciliated cells of the epithelial airways.

Conclusion: Our data demonstrate the NBCe1 co-transporter is expressed in airway epithelium and plays an important role in bicarbonate secretion. The observation that bicarbonate secretion increases with age suggest that the mucin viscoelasticity might also change and, the impact of decreased bicarbonate secretion could be more deleterious in adults that infants.

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PLC-δ1 is a new partner of CFTR in pulmonary epithelial cells : role and physiological consequences

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Background: In cystic fibrosis epithelial cells, a calcium deregulation occurs with (i) an increase of the SERCA pump (Phillipe *et al*., 2015) and the IP_3 receptors (Antigny *et al*., 2008) activities, (ii) a decrease of the PMCA activity (Phillipe *et al*., 2015) and (iii) a decrease of the calcium fluxes mediated by the TRPC6 (Antigny *et al.*, 2011) and TRPV6 channels (Vachel *et al*., 2015). This increase of calcium responses contributes to the CF phenotype.

Objective: Vachel *et al* reported an increase of TRPV6 activity in CF epithelial cells, with no difference on the protein expression between CF and non CF cells. The aim of this study is to understand why TRPV6 activity is increased in pulmonary cells in CF and what are the repercussions on CFTR activity. For this, we studied (i) the expression and modification of proximity between common partners of TRPV6 and CFTR and (ii) the impact of these partners on CFTR activity. Among the proteins that regulate TRPV6, we focused on PLC-δ1 that negatively regulates TRPV6 by cleaving PIP_2 at the plasma membrane.

Methods: We used human polarized pulmonary epithelial cell lines 16HBE14o (wt-CFTR) and CFBE41o- (F508del-CFTR). The expression and the interaction between cellular partners of CFTR and TRPV6 (ex : PLC-δ1, AnnexinA2, S100A10, and PIP2) are obtained by Western blot and by the proximity ligation assay using immunofluorescence. CFTR activity is measured in Ussing chambers.

Results and discussion: In CF cells, PLC-δ1 expression is 50% decreased compared to their counterpart non CF cells. Thus, the cellular proximity between the PLC-δ1 and CFTR is disturbed in F508del-CFTR cells where the CFTR-PLC-δ1 proximity is 50% decreased compared to wt-CFTR cells, showing that PLC-δ1 protein is a partner of CFTR in non CF epithelial cells. On the other hand, PLCs take part to CFTR current (Ivonnet *et al*., 2015) as PLC inhibitor U73122 triggered a 40% decrease of CFTR current in wt-CFTR polarized cells. We would like to understand if TRPV6, PLC-δ1 and CFTR belong to a same cellular complex, as we know that PLC-δ1 increases CFTR activity and decreases TRPV6 activity. TRPV6 deregulation will also be lightening with the AnnexinA2-S100A10 complex that is regulating both TRPV6 and CFTR at the plasma membrane. Does this complex drive more active TRPV6 in CF cells ? Moreover, does the PLC-δ1 have a role in this regulation ?

Conclusion: PLC-δ1 and CFTR proximity is disturbed in CF pulmonary epithelial cells. This data could explain how TRPV6 and CFTR are regulated in CF cells.

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Effect of exocrine dysfunction on the endocrine pancreas

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Background: The exocrine and endocrine part of the pancreas work in close interaction with each other in which the CFTR Cl^- channel plays an essential role by regulating the secretory processes. Although, the role of the channel and the relation between the exocrine/endocrine pancreas under certain pathological conditions is not completely known, therefore our **aim** in this study is to investigate the role of CFTR in the case of diabetes and/or pancreatitis and to study the effect of exocrine inflammation on pancreatic function both in control and diabetic mice.

Method:**.** Intra-interlobular pancreatic ductal fragments were isolated from FVB/N and CFTR knock out mice by enzymatic digestion. Pancreatic ductal HCO_3^- secretion was measured by the alkali load technique using fluorescence microscopy, whereas pancreatic ductal fluid secretion was examined by videomicroscopy. Pancreatitis was induced by intraperitoneal injection of cerulein and disease severity was assessed by measuring laboratory and histological parameters. Diabetes was induced by ip. administration of streptozotocin and disease development was confirmed by measurement of serum level of glucose and insulin.

Results:The absence of CFTR decreased the fluid and HCO_3^- secretion of the ductal cells, worsened the severity of pancreatitis and decreased the number of α and β cells. Exocrine inflammation decreased the serum level of insulin and glucagon and also strongly impaired exocrine secretion. The presence of diabetes only slightly inhibited HCO_3^- secretion, and increased the fluid secretory rate in the ducts.

Conclusion: Our results suggest that the CFTR Cl^- channel plays a key protective role both in the exocrine and endocrine pancreas and that exocrine inflammation is probably involved in the endocrine secretory defects, however further investigations are needed to confirm this hypothesis.

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Borrowing epithelial chloride transporters and channels to help understand insulin secretion in CFRD

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Insulin-secreting β-cells and epithelial cells share the expression of many Cl^- co-transporters and channels, including the Cl^- loader *Nkcc1* and the Cl^- channels *Ano1*, *Cftr* and *Lrrc8a-e*. These cells are polarized and maintain an outwardly directed Cl^- gradient, which in epithelial cells supports fluid secretion, regulates cell volume, size, growth and proliferation. In β-cells, such Cl^- gradient regulates cell volume and insulin secretion. Although the involvement of *Nkcc1*, *Ano1*, *Cftr* and *Lrrc8a-e* in transepithelial ion transport and fluid secretion is well recognized, their impact and purpose in islet function, together with β-cell proliferation and insulin secretion remains poorly defined, for the most part for *Cftr* and *Ano1*. This is a clinical relevant issue, because with improved treatment and increased survival, approximately 45-50% of patients with cystic fibrosis (CF) develop CF-related diabetes (CFRD) by the time they reach the fourth decade of life. CFRD is a complex metabolic syndrome, primarily thought to result from β-cell injury due in part to pancreatic exocrine damage and lipofibrosis, which is mainly characterized by defective insulin secretion and fasting/fed or intermittent hyperglycemia, that increases mortality by worsening lung function and nutritional status. Current data from our group strongly suggests, that important factors in the progression of CF to CFRD include a combination of events related to reduced islet density and altered cellular composition. In particular, *i*) a very early and significant decrease in β-cell mass up to 50% or more, in comparison to age-matched young infant controls, *ii*) a lower β-cell proliferation index and neogenesis, and *iii*) the presence of islet inflammatory cell infiltrates, independently of the severity of the exocrine lesion and in the absence of amyloid deposits, which result in increased dysfunction of the endocrine unit of the pancreas among other unknown or poorly defined mechanisms. One of these mechanisms relates to the ionic regulation of the secretory response and β-cell plasma membrane depolarization, the event that makes possible the initial insulin secretory burst in response to glucose and nutrients. Although the primary mechanism involved in this response is the one triggered by the closure of ATP-sensitive K^+ channels (K_{ATP}), recent evidence supports the involvement of *Cftr* and *Ano1* in the regulation of depolarizing outwardly-directed Cl^- fluxes, therefore the electrical properties of β-cells and insulin secretion. The aim of this study was to pharmacologically dissect the functional role and relative participation of Cl^- channels in the regulation of the secretory response in primary rodent pancreatic islets and clonal β-cell lines. Our results suggest that *Cftr*, *Ano1* and *Lrrc8a-e* participate in the insulin secretory response, independently of K_{ATP} channels, and reliant on Cl^- gradients, mostly set by *Nkcc1* function.

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Pancreatic ductal fluid secretion is significantly reduced in newborn ferrets and pigs with cystic fibrosis

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Introduction: Cystic fibrosis (CF) is a lethal genetic disease affecting several organs, including the pancreas. Several animal models are available to study the CF related pancreatic tissue damage although they have clear limitations. Recently a cystic fibrosis transmembrane regulator (CFTR) knockout ferret and pig model have been generated.

Aim: We aimed to characterize the fluid and bicarbonate secretion of CF and wild type (WT) ferret and pig pancreatic ducts.

Methods: Intra/interlobular pancreatic ducts were isolated from newborn CF and WT ferret and pig pancreata. In the ferret model the expression of CFTR was detected by immunohistochemistry and resting pH, buffer capacity and Cl^-/HCO_3^- exchange activity were evaluated by microfluorometry. Fluid secretion of ducts from CF and WT ferrets and pigs were examined by videomicroscopy.

Results: CFTR was expressed on the luminal membrane of the WT ferret pancreatic ducts. The resting intracellular pH of pancreatic epithelial cells was 7.17±0.08 in ferrets. Concerning bicarbonate influx mechanisms, functionally active sodium/hydrogen exchangers and sodium/bicarbonate cotransporters were detected in WT pancreatic ducts in ferrets. Anion exchanger activity was measured by $NH4Cl^-$technique and inhibitory stop methods in CF ferret and WT ducts. Our results indicate that the bicarbonate secretion is significantly decreased in CF ferret ducts compared to WT. Videomicroscopy revealed a significant increase in fluid secretion to HCO_3^{---} and to 5μM forskolin and 100 μM IBMX stimulation in both WT pig and WT ferret ducts. In CF ferret and pig ducts increase of the fluid secretion were not detected during the stimulation period with 5μM forskolin and 100 μM IBMX. We repeated our measurements in HEPES buffered solutions with forskolin and IBMX stimulation and our results revealed that fluid secretion did not increase in CF ferret and pig ducts during the stimulation period. While in WT ferret and pig ducts stimulation of the fluid secretion occurred in both cases

Conclusion: Major epithelial ion transporters are expressed in WT ferret pancreatic ductal epithelial cells. Our results indicate that in CF ferret the pancreatic ductal fluid and bicarbonate secretion is significantly decreased. Concerning our data, absence of the CFTR can lead to decreased or completely abolished pancreatic ductal fluid secretion. These demonstrate the crucial effect of the loss of function of CFTR in pancreatic ductal epithelial cells. Our interesting results also revealed the importance of studying pancreatic ductal secretion of these new CF animal models more closely.

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Conditional deletion of *Nedd4-2* in lung epithelial cells in adult mice leads to distal lung remodeling associated with elevated ENaC activity

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In the airways, the epithelial Na^+ channel (ENaC) contributes to airway surface liquid homeostasis by constant Na^+ absorption. In cystic fibrosis (CF), the missing inhibitory regulation of ENaC by the cystic fibrosis conductance regulator (CFTR) leads to Na^+ hyperabsorption, airway surface dehydration and impaired mucociliary clearance (MCC). The ubiquitin ligase Nedd4-2 is one of the major regulators of ENaC surface expression in lung epithelia by mediating ubiquitination, endocytosis and lysosomal degradation. Previous studies showed that constitutive knock-out of *Nedd4-2* in the lung resulted in increased ENaC protein levels, massive neutrophilic inflammation in distal airspaces and premature death 2-3 weeks after birth.

We aimed to study the role of Na^+ hyperabsorption in the adult lung *in vivo* independent of lung development by generating a mouse model with conditional deletion of Nedd4-2. We, therefore, crossed mice carrying *Nedd4-2* flanked by *loxP* sites (*Nedd4-2*$^{fl/fl}$) with CCSP-rtTA2^S-M2/LC-1 mice enabling tight doxycycline-induced rtTA-mediated expression of Cre recombinase producing deletion of *Nedd4-2* in alveolar type 2 cells and club cells of the conducting airways. After 2 weeks of induction increased levels of ENaC protein could be readily observed and *Nedd4-2* deficient mice showed increased ENaC-mediated Na^+ currents across freshly excised airway tissues and airway surface liquid (ASL) depletion on primary airway cultures. After 3-4 month of doxycycline induction we observed spontaneous mortality associated with severe weight loss and hypoxia resulting in an overall mortality of 70% at 4 months in conditional *Nedd4-2*$^{-/-}$ mice. However, consecutive analyses of lung morphology revealed patchy fibrosis of the alveolar interstitium associated with inflammatory infiltrates. We also evaluated cell type composition, i.e. numeric densities of CCSP-positive club cells, Tubulin-positive ciliated cells, and AB-PAS-positive goblet cells along the tracheobronchial tree. These studies showed epithelial remodeling of the peripheral airways characterized by a decrease in club cells and increase in ciliated cells and goblet cells in distal and terminal airways of conditional *Nedd4-2*$^{-/-}$ compared to control mice.

In summary, conditional deletion of *Nedd4-2* in lung epithelial cells of adult mice results in dysregulation of ENaC activity and ASL height, as reported for CF airways, leading to fibrotic lesions in the peripheral lung. We speculate that impaired MCC in the peripheral airways may trigger repeated injury and inflammation and contribute to remodeling of distal airspaces in conditional *Nedd4-2*$^{-/-}$ mice.

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Upper airway phenotype in the β-ENaC overexpressing mouse

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In CF airway epithelium, defective CFTR-dependent anion (Cl^- and bicarbonate) transport and elevated ENaC-dependent Na^+ entry are the main causes of airway surface liquid dehydration, the initiating event of CF airway disease pathogenesis. The critical role of increased sodium conductance in the development of CF lung disease is strongly supported by the *scnn1b-Tg* (βENaC overexpressing) mouse engineered to mimic the hyperactivity of ENaC channels selectively in lower airways.

Interestingly, using *in vivo* transepithelial potential difference (PD) measurements, we observed a significant increase (+50%) in Na^+ absorption across the nasal mucosa of *scnn1b-Tg* heterozygous (Tg/+) compared to WT mice. CFTR-dependent Cl^- transport was not affected by ENaC hyperactivity in Tg/+ mice nose. To determine if increased Na^+ absorption in Tg/+ mice nose can be, at least partly, explained by difference in sensitivity to proteolytic activation of ENaC channels, we applied, by local deposition onto the nasal mucosa, the protease inhibitor aprotinin and we compared amiloride response in presence of aprotinin alone to those obtained after maximal activation of ENaC channels by perfusing the nasal mucosa with trypsin. Ongoing analyses will determine if Tg/+ mice express different populations of ENaC channels in the nasal epithelium compared to WT mice.

We also sought to determine if the elevated transepithelial Na^+ transport is directly linked to the expression of the Scnn1b transgene in the upper airways. In this mouse model, the airway specific Clara cell secretory protein (CCSP) promoter was used to limit the expression of Scnn1b subunit transgene to the lower airways. Although its expression has long been thought to be restricted to the lower airways, we detected significant endogenous expression of the *CCSP* gene in the mouse nasal epithelium, even though at a lower level than in the trachea and the lung. Additionally, we detected by RT-PCR a fragment of the SV40 promoter controlling the expression of the transgene in fresh nasal epithelium, confirming that the Scnn1b-Tg construct is expressed in the nasal epithelium of Tg/+ mouse. This fragment was also detected in primary cultured mouse nasal epithelial cells (MNEC), suggesting that MNECs also express the hyperabsorptive phenotype of the native tissue.

The volume of fluid on apical surface of murine nasal epithelial cells depends on the balance between CFTR-dependent Cl^- secretion and ENaC-dependent Na^+ reabsorption. The apical fluid at the apical surface of confluent and fully differentiated MNEC monolayers was visualized using light microscopy and was collected and weighted as a measure of volume. Virtually no fluid was visualized on the surface of Tg/+-MNEC monolayers. The volume of apical fluid was significantly reduced in MNEC cultures from Tg/+ mice compared to WT mice.

Taken together, our results support the view that the CF-like β-ENaC overexpressing mouse model presents with an upper airway phenotype concordant with an expression of the CCSP-controlled Scnn1b transgene.

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Decreased VIPergic innervation in duodenum tissue of young C57Bl/6 CF mice

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The major physiological agonist of the Cystic Fibrosis Transmembrane conductance Regulator (CFTR) chloride channel is the Vasoactive Intestinal Peptide (VIP), a 28-amino acid neuropeptide that functions as a neuromodulator and neurotransmitter secreted by intrinsic neurons innervating exocrine glands. VIP is a potent vasodilator and bronchodilator that also regulates exocrine gland secretions, contributing to local innate defense, by stimulating the movement of water and chloride across intestinal and tracheobronchial epithelium. Previous studies have demonstrated that normal skin, intestinal and nasal mucosa have rich intrinsic neuronal networks for VIP secretion around exocrine glands, in contrast to absent or minimal VIPergic innervation of CF tissues. These early findings suggest a critical role for VIP in CF development. Our lab has since confirmed, *in vitro* and *in vivo,* the need for chronic VIP exposure to maintain functional CFTR chloride channels at the cell surface of airways and intestinal epithelium as well as normal exocrine tissues morphology.

In the present study, we have investigated changes in VIP in exocrine tissues of 8- to 17-week-old C57Bl/6 CF mice (n=5) compared to same age wild-type tissues. Additionally, we have investigated changes in VIP innervation. Paraffin-embedded tissues were sectioned and H&E stained for pathological assessment, observed under light microscopy and imaged for semi-quantification. VIP was revealed by immunohistochemistry and the amount of VIP was semi-quantified by two blinded investigators using arbitrary scale. To study changes in VIP innervation, we used the whole-mount immunofluorescence method where fresh duodenum tissues (1cm sections) from 8-week old wild-type and CF mice (n=5) were immunostained and imaged with 3D confocal microscopy reconstruction. Lastly, we measured the median VIP concentration in 8-week-old WT and CF duodenum homogenates (n=5) with ELISA. All experiments were repeated twice.

Our results show a strong reduction in VIP in young CF mice with minimal signs of inflammation or tissue damage. VIP reduction continued to be observed in advanced disease animals (17-week old). Interestingly, a 44% to 54% reduction in VIP was found in CF sweat glands which are free of inflammation at all ages. In other tissues, we found that the VIP signal was reduced in the lung (44% and 24%), salivary glands (7.34% and 42%), duodenum (48% and 38%), the endocrine (59.43% and 35.83%) and exocrine pancreas (24.68% and 43.11%) at 8- and 17-week old respectively. Moreover, we observed a strong reduction in VIP innervation in the duodenum of CF mice at the mucosa and circular muscle layer with fewer fine axons in villi but no decrease in cell bodies compared to WT. ELISA results confirmed a decrease in VIP concentration in the 8-week-old CF duodenum tissue homogenates (median: 1616pg/ml and 888pg/ml in WT and CF respectively, n=5).

In conclusion, our data suggest that the low amount of VIP found in CF corresponds to reduced VIPergic innervation of exocrine tissues. Moreover, we propose this represents an early defect in CF and constitutes an aggravating factor for disease progression that needs to be further investigated at the molecular level.

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BMI-1 transduced basal cells as a renewable resource for air liquid interface cell culture models of cystic fibrosis

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Cell models of cystic fibrosis (CF) comprising air-liquid interface (ALI) cultures of primary airway cells from CF donors enable the study of many aspects of epithelial and mucociliary biology in CF as well as the evaluation of therapeutics in relevant model systems. ALI cultures are established with basal cells obtained by nasal or bronchial brushings which then differentiate into a pseudostratified epithelium. A major problem with this method is that basal cells, the progenitors of the different airway epithelial cell types, have a limited capacity for expansion requiring repeated brushing of donors. Conditionally reprogrammed cultures are one solution but have practical limitations in requiring feeder layers. We have previously described a simple alternative approach to extended passaging in which basal cells are transduced with *BMI-1,* an anti-senescence gene. In this study, we have now evaluated the utility of BMI-1-transduced basal cells as an in vitro model of cystic fibrosis.

Normal human bronchial epithelial cells (NHBE), and CF epithelial cells (CFBE) from a patient homozygous for the 3849+10kb C->T mutation, were transduced with a lentiviral vector encoding BMI-1 at multiplicities of infection (moi) of 1, 4 and 16 then maintained in submerged cell culture. Both types of BMI-1 cells maintained their rate of cell division for at least 7 passages over a 50-day period while, as expected, untransduced primary cells showed a slower growth rate after two passages. CFTR mRNA expression levels in both NHBE-BMI-1 and untransduced, primary NHBE cells (both passage 5) in well-differentiated ALI culture was approximately 1,000-fold higher than in their respective, submerged basal cell cultures as demonstrated by quantitative RT-PCR analysis. Western blot analysis of CFTR protein showed that both CFTR C and B bands were detectable in NHBE-Bmi-1 and primary cells from ALI cultures.

The CFBE-BMI-1 cells contain the 3849+10kb C->T mutation, which creates an internal intronic splice site and formation of a larger transcript, leads to complete loss of CFTR protein. This mutation, however, is associated with a mild phenotype of CF as a proportion of the transcript are correctly spliced. We confirmed by RT-PCR analysis that both primary CFBE cells on ALI (from feeder layer cultures) and CFBE-BMI-1 cells with the 3849+10kb C->T mutation showed a mixture of normal and mutant transcripts and that there was a small but detectable forskolin inducible short circuit current which was more apparent with NHBE cells. Thus, CFBE-BMI-1 cells homozygous for the 3849+10kb C->T mutation, a very rare genotype, provide, with carefully planned expansion regimes, an almost unlimited supply of cells for biological and therapeutic studies without the need to revisit the original donor.

In conclusion BMI-1 transduction provides an alternative approach to expanding epithelial basal cell cultures in CF model systems that replicate the molecular and physiological features of CF primary cells in ALI cultures.

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May the primary human nasal epithelial cell cultures be considered as a biomarker to predict the clinical efficacy of CFTR modulators?

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Evaluation of CFTR modulators requires a physiologically relevant predictive biomarker to test their efficacy in restoring CFTR function. The *in vitro* reconstituted human respiratory epithelium from primary human nasal epithelial (HNE) cells is a promising system enabling the prediction of a drug activity and toxicity in patients. In our previous study we evaluated the correction of CFTR processing and function by lumacaftor (VX-809) and tezacaftor (VX-661) in primary HNE cell cultures obtained from F508del homozygous patients or carrying CFTR genotypes displaying a wide spectrum of CFTR activity. We showed in a pilot study that the correction of CFTR activity in HNE cultures potentially constitute an *in vitro* predictive biomarker for the clinical efficacy of CFTR modulators [1].

The aim of the present study is to confirm these results and get further insight into the predictive value of the HNE culture by assessing the correlation between the level of CFTR functional correction in HNE cultures and the patients' clinical improvement upon Orkambi treatment.

HNE cell cultures were isolated by nasal brushing before lumacaftor/ivacaftor combination (Orkambi) treatment. Cells were conditionally reprogrammed and grown at air-liquid interface for differentiation to measure the CFTR-dependent Cl^- secretion by addition of forskolin followed by CFTR potentiation with acute ivacaftor (VX-770) (10 µM) addition in the Ussing chamber ($\Delta Isc_{F/I+V}$). Percentage of the predicted forced expiratory volume in 1 sec ($\%FEV_1$); sweat test; response to low chloride/Isoproterenol solution (LCIso) measured by nasal potential difference (NPD) and *ex vivo* response to Forskolin (Fsk) measured by short circuit current on intestinal biopsies (ICM) (Fsk-ICM) were measured before treatment and at 6 months of Orkambi treatment.

Mean correction of CFTR activity by VX-809 was 10.3% (SEM = 3.8). $\Delta Isc_{F/I+V}$ correction was significantly correlated to FEV_1 modification by 6 months Orkambi treatment (n = 22; p = 0.04). The mean value of $\Delta Isc_{F/I+V}$ correction was significantly higher in the group of patients with increase in FEV_1 by 6 months by more than 5% (n = 7; mean $\Delta Isc_{F/I+V}$ improvement by 23% (6.2)) *versus* the group of patients with FEV_1 variation below 5% (n = 15, mean $\Delta Isc_{F/I+V}$ = 4.3% (3.9)), (Mann Whitney test; p = 0.03). $\Delta Isc_{F/I+V}$ correction was also significantly correlated to Fsk-ICM modification by 6 months Orkambi treatment (n = 7; p = 0.044). By contrast, no correlation was demonstrated between $\Delta Isc_{F/I+V}$ correction and either LCIso or sweat test modification.

Primary HNE cultures could be considered as a biomarker to predict the clinical efficacy of CFTR modulators and may constitute a tool for personalized therapy in CF.

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Nasal epithelial cultures from patients homozygous for F508del and not responsive to ORKAMBI® can show positive in-vitro responses to other modulator combinations

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There is growing acceptance of the concept that in-vitro assays of cells expressing mutant CFTR may inform therapeutic strategies. However, it is not yet clear which in-vitro assays will exhibit the greatest utility in directing effective clinical care. For CFTR mutants that are synthesized and trafficked to the cell surface like G551D-CFTR there is a strong correlation between the efficacy of channel potentiators in heterologous expression systems and native tissues. On the other hand, the efficacy of modulators of mutant F508del-CFTR protein assembly and trafficking is dependent on the cell type (Pedemonte, 2010). To add another layer of complexity, *in-vitro* efficacy of F508del-CFTR modulators in primary tissue also appears to be dependent on the donor. Importantly-studies by Pranke et al (2017) showed that CFTR chloride channel responses observed in patient derived nasal epithelial cultures of varying genotypes (including F508del homozygous and heterozygous) correlate with certain clinical outcomes measured after initiation of ORKAMBI® treatment. Hence, the nasal epithelial culture model may have utility in predicting those who are responsive to ORKAMBI® and who may not be responsive. In the current studies- we asked if this nasal epithelial model system of patient-specific responses could be used to identify new compounds that may be effective for those who are not responsive to ORKAMBI®.

We studied nasal epithelial cultures from multiple donors to the CFIT program at SickKids hospital, Toronto who were F508del-CFTR homozygous (n=6). The relative response to a selection of correctors, including VX-809 and 3 novel correctors, was studied by Ussing Chamber and mutant CFTR protein expression was quantified by Western blotting. Using the thresholds of 50% Wt forskolin mediated Ieq ($\mu A/cm^2$) to signify response, we determined that cultures from 1/6 F508del-CFTR individuals were responsive to correction with lumacaftor and potentiation with ivacaftor ($-5.5\mu A/cm^2$). Of the remaining cultures from 5 individuals- we detected distinct modulator combinations that were effective in rescuing residual F508del function to 50% or greater of Wt-CFTR (ranging from -4.4 to -- 26.1 $\mu A/cm^2$).

These findings support the concept that modulators of misprocessed mutant CFTR, exhibit donor specificity and that alternative modulator combinations can be identified that are effective in restoring F508del-CFTR function to a range that may be therapeutically relevant. These findings support our future goals to develop a platform to enable preclinical trials to identify the optimal modulator using donor specific nasal epithelial cultures.

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P49

Nasal epithelial cells expansion and reprogramming with the anti-SMAD protocol to evaluate CFTR rescue for precision medicine

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There is an increasing availability of small molecules (potentiators, correctors, amplifiers) to rescue mutant CFTR. Such compounds are an opportunity for a personalized approach to the treatment of cystic fibrosis patients. The sensitivity of the various CFTR mutants to single molecules or combination of molecules needs to be determined to find the best treatment for each patient. This goal can be achieved by collecting nasal epithelial cells with a nasal brushing procedure. However, cells obtained by nasal brushing are mostly terminally differentiated with a limited proliferating ability. We adopted a recently published method based on the combination of three molecules DMH-1, A-83-01, and Y-27632 (Mou et al., Cell Stem Cell 19:217-231, 2016), which suppress SMAD signaling supporting the proliferation of basal stem cells. Using the anti-SMAD protocol we could generate up to 120 millions of cells from a single brushing in 4-5 passages. Such cells can be conveniently stored in frozen aliquots. When seeded on porous membranes (Snapwell inserts), cells can be reprogrammed with a specific culture medium to obtain differentiated epithelia with mucociliary properties. We estimate that 150-200 epithelia can be obtained with a single brushing. By Ussing chamber experiments, we could confirm activity of F508del-CFTR correctors, with an extent of rescue similar to that measured in bronchial epithelial cells (control: 1.3 ± 0.1 μA; VX-809: 3.4 ± 0.5 μA). Interestingly, nasal epithelial cells consistently showed a larger ENaC function compared to bronchial epithelial cells generated with the same protocol. Indeed the amiloride-sensitive current was 5.9 ± 1.3 and 0.8 ± 0.1 μA in nasal and bronchial cells, respectively. This difference may suggest an intrinsic higher ENaC expression in cells of nasal origin. In conclusion, our findings confirm the anti-SMAD protocol as a convenient method for precision medicine in cystic fibrosis. We are currently using this approach to study the pharmacological sensitivity of cells with rare mutations.

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P50

Characterisation of primary paediatric nasal epithelial cells as a model system to investigate TMEM16A as a therapeutic avenue in CF

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Background: Modulation of the calcium activated chloride channel (CaCC), TMEM16A, has potential to restore airway surface liquid homeostasis and represents a promising target for CF therapy. Robust models are required to explore this further particularly in children where disease processes occur early. Primary bronchial epithelial cell (PBEC) cultures have been pivotal in CF drug discovery, however their acquisition from children is invasive. Primary nasal epithelial cell (PNEC) sampling is less invasive, but paediatric PNEC characterisation of TMEM16A remains limited.

Methods: Nasal brushings were sampled from children with and without CF and cultured using an air liquid interface (ALI) model to provide fully differentiated cultures. Histological assessment, immunofluorescence techniques and transepithelial electrical resistance (TEER) measurements were used to assess epithelial characteristics. Ussing chamber experiments were performed to assess short circuit current (Isc) responses to channel agonists and inhibitors. Expression of relevant ion channels were investigated using quantitative real-time polymerase chain reaction (q-PCR). Paired analyses of PNECs and PBECs isolated from the same donors provided comparative assessment and validation for PNECs as a representative model.

Results: PNECs demonstrated airway epithelial characteristics with evidence of ciliated, differentiated cells on histology and immunofluorescence. TEER measurements were comparatively higher in CF cultures. At day 28 ALI, CF PNECs demonstrated the highest TEER (1537±772 ohm.cm^2 n=12 donors), which was significantly greater than CF PBECs (817±339 ohm.cm^2 n=7; $p < 0.05$).

The CFTR activator forskolin produced a robust Isc increase in all non-CF donors, which was blocked by CFTRinh-172, with no significant differences found between non-CF PNECs and PBECs. Although not significant, the mean Isc reduction in response to the epithelial sodium channel inhibitor amiloride was greater in PNECs compared to PBECs and more pronounced in non-CF cultures.

Addition of UTP to activate CaCC did not show any significant differences in Isc between PBECs versus PNECs nor between CF and non-CF donors (non-CF PNECs: 5.0±4.1 n=6 donors; non-CF PBECs: 2.5±0.5 n=4; CF PNECs: 3.0±3.0 n=5; CF PBECs: 2.3±1.3 n=3; measured in μA/cm^2). The TMEM16A inhibitor CaCCinh-A01 reduced the UTP-induced Isc response by 56% in CF PNECs (n=3 donors). To further characterise TMEM16A function, the putative small molecule TMEM16A activator Eact demonstrated a 2-fold increase in Isc in non-CF PNECs versus CF (2.5±0.3 versus 1.0±0.4 μA/cm^2, $p < 0.05$). Overall there was notable inter-donor variability in Isc responses in all PNECs. Functional assessment of TMEM16A was complemented by immunofluorescence and q-PCR, with IL-4 mediating a significant up-regulation of expression and function.

Conclusions: PNECs represent a valid paediatric CF research tool. Furthermore, we provide evidence that TMEM16A is functionally expressed in the paediatric CF airway epithelium which will facilitate investigation of TMEM16A modulation as a potential CF therapeutic avenue. The apparent inter-donor variability in PNECs, however, suggests there is a need for a personalised approach to enable individual profiling of ion transport. This will be invaluable for targeted investigation of potential novel therapies in children with CF.

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P51

Development of a robust protocol for gene-editing human pluripotent stem cells

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Gene-editing technologies such as TALENs and CRISPR/Cas9 have shown great promise in providing numerous knock-out/knock-in models that bring new insights to the CF field. Additionally, refined differentiation protocols of human pluripotent stem cells (hPSCs) towards several cell/tissue lineages highlight hPSCs as a valuable resource for modelling numerous multiorgan diseases, and a very powerful platform for tissue-specific drug screening in CF. Therefore, it is logical that customized gene-edited hPSC lines would greatly facilitate the improvement of our understanding of CF pathogenesis as well as drug discovery. However, the gene-editing techniques are still very challenging to perform on hPSCs, partially due to low efficiency of homology-directed repair (HDR). Therefore, our goal was to establish a robust and fast gene-editing protocol for hPSCs.

We optimized a protocol for gene-editing hPSCs by minimizing time and costs required to produce correctly edited hPSC lines. It includes: 1) highly efficient transfection method conditions, 2) rapid cell population screening for correct cleavage by designer nucleases, and 3) integration of the desired mutation/correction through HDR; 4) manual clonal isolation and rapid selective screening to identify and confirm correctly modified clones. This protocol was developed on a non-CF human embryonic stem cell (hESC) line (CA1) to produce a homozygous delF508 CF-hESC line using TALENs. Additionally, we applied this protocol to correct 3 CF-human induced (iPSC) lines homozygous for a nonsense mutation, W1282X, using CRISPR/Cas9. 1) These hPSC lines were nucleofected with plasmids encoding either *CFTR-exon11*-targeting TALENs or *CFTR-exon23*-targeting CRISPR/Cas9, in addition to a short single-strand oligonucleotide harbouring the delF508 mutation or the correct WT sequence, respectively. 2) 5-8 days post-nucleofection, the T7 Endonuclease I assay showed 16-37% cleavage at correct sites in all cell populations, suggesting TALEN and CRISPR activities in all hPSC lines. 3) Subsequently, a screening method using allele-specific PCR (AS-PCR) selective for the delF508 mutation and WT sequence was optimized for the hESC and iPSC lines, respectively, which indicated the desired sequence in all hPSC populations. 4) Clonal isolation was then manually performed and one round of clones per cell line was screened by AS-PCR, 2-6 days post-isolation. Identified positive clones were subjected to sequencing analysis which confirmed 1.9% (1/52 AS-PCR screened) pure homozygous delF508 CF-hESC line when using TALENs, in addition to 0.9% (1/110) pure heterozygous corrected, and 0.7% (1/146) and 6.4% (3/47) pure homozygous corrected iPSC lines, confirming that our optimised protocol could a produce a homozygous delF508 CF-hESC from a healthy-hESC line, and we could also correct 3 CF-iPSC lines, both in a 3-6 weeks period. These cell lines are currently being characterized in terms of their pluripotency, chromosomal stability and ability to differentiate into a specific cell lineage.

In summary, we have developed a robust and fast protocol for gene-editing hPSCs. This approach should facilitate the production of new "in-house" CF and corrected hPSC lines that will qualitatively and quantitatively expand the available range of tissue-specific models for CF pathogenesis research, and significantly improve the personalized medicine field in CF.

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P52

Development of gene edited cell models to study Cystic Fibrosis

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Cystic Fibrosis (CF) is the most common chronic and life-threatening genetic disease affecting the lungs and it can be caused by nearly 2000 different mutations in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene. Although, two drugs targeting mutant CFTR have been approved for clinical use two major problems still persist: 1) neither of these modulators are sufficiently effective to be used as stand-alone treatments; 2) they only benefit approximately 50% of the CF-patients [1]. Thus, it is crucial to find more efficient and broader therapeutic approaches for the rarer mutations. The use of cellular models provides valuable information but most of the existing cell lines rely on overexpression of CFTR rather than on endogenous expression of CFTR.

To address this issue, we present data from two gene editing methods aimed at developing isogenic cellular models homozygous for different CF-mutations, using an epithelial cell line - Calu3 - derived from lung adenocarcinoma. Both methods are being developed in parallel and will be compared.

The first method uses Cas9/gRNA expressed from a plasmid and a piggyBac donor to introduce the mutations and allow selection of edited cells. We designed three different gRNA to have minimal off-target effects, all of which were successfully validated in Calu-3 cells using T7 Endonuclease assay.

The second method uses Cas9/gRNA ribonucleoproteins (RNPs) and single strand DNA (ssDNA) oligonucleotide donor, which shows much higher HDR editing efficiency and reduces off-target effects [2]. In this case, three different RNA molecules, designed for the same target sequences as above, were assembled with high fidelity Cas9 to form the RNPs, delivered into Calu3 cells and successful validated with the T7 Endonuclease assay. All three gRNAs showed evidence of double stranded break (DSB) formation, but one of the gRNAs worked particularly well in both systems. This gRNA has been selected for co-transfection with a ssDNA donor (for use with RNPs) or a piggyBac donor (for use with Cas9/gRNA expression plasmid) to identify the most effective method to generate the mutant cell lines. In the first instance, we will create a cell line with F508del mutation for which a piggyBac donor has already been constructed. Three different ssDNA molecules will be tested with the RNPs with the mutation near the 5' end, the middle, or near the 3' end.

In summary, two different genome editing methods have been established for the generation of isogenic homozygous mutant CF cell lines and work in progress includes the design of new gRNAs to target other mutations (including rare ones). Their characterization at the cellular and molecular level will lead us to our ultimate goal of finding disease signatures for CF that may result in novel therapeutic outcomes.

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P53

Identification of (TECC-24) assay and cell culture conditions to assess therapeutic rescue of PTC CFTR variants in gene-edited 16HBE14o$^-$ cell lines

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Effective therapeutics are a serious unmet need for CF patients with nonsense variant alleles. Finding effective treatments for these patients is hampered by insufficient supply of primary hBE or hNE cells homozygous for these rare CFTR variants. The shortage of primary cells makes the development of viable alternative models imperative for the discovery and characterization of novel therapeutics for particular mutations in their native genetic context.

Previous efforts using PTC CFTR cDNA expressing FRT cells have failed to identify read-through compounds with significant activity in primary cells. We screened 200k small molecules in a CFTR cell-surface expression assay in stable FRT cell lines expressing CFTR PTC variants from cDNA. Hit confirmation by electrophysiology yielded over 100 hits that marginally, but significantly (>3SD) increased the CFTR-mediated Cl^- transport activity in these cells. This functional rescue was assumed to be indicative of promotion of PTC readthrough by the compounds. However, no rescue of CFTR activity could be detected when hits were profiled (mRNA, protein, function) in primary hBE or the gene-edited 16HBE14o$^-$ cell lines expressing CFTR PTC variants from the native gene locus. To identify compounds for which readthrough activity and rescue of CFTR function translates to patient-derived hBE/hNE cells, we are currently developing novel HTS-suitable cell lines that express CFTR from the endogenous gene locus.

Using CRISPR/Cas9 gene editing techniques, we derived cell lines homozygous for G542X or W1282X from 16HBE14o$^-$ cells (Dieter Gruenert, UCSF). These cell lines are currently being evaluated for usability in HTS and electrophysiological assays. Here, we are describing cell culture and experimental assay conditions suitable for the assessment of rescue of CFTR function in these cell lines. Preliminary data with reference compounds will be presented side-by-side with data obtained from another immortalized hBE cell line, homozygous for G542X (CF7T, Scott Randell, UNC Chapel Hill).

Preliminary results are summarized in the table below. 16HBE cells were cultured on Transwell filters for 6-7 days in MEM media at a liquid-liquid interface (LLI) and CFTR Cl^- transport activity was recorded in the presence of a Cl^- gradient (ΔCl^- = 137 mM; bl. > ap.). CF7T cells were cultured on Transwell filters for 4-5 weeks in Vertex ALI differentiation media and CFTR Cl^- transport activity was recorded in symmetric buffer solutions.

Treatment (96h, 37oC)	16HBE14o- (WT/WT)	16HBEge (W1282X/W1282X)	16HBEge (G542X/G542X)	CF7T (G542X/G542X)
	µA/cm^2 (Inh172)	µA/cm^2 (Inh172)	µA/cm^2 (Inh172)	µA/cm^2 (AUC/min)
Vehicle	40-150	0	0	0.3
G418		1.7 (1.3% WT)	3.5 (9.5% WT)	1.0
G418 +VX-809		2.2 (1.7% WT)	4.5 (12.2% WT)	1.5
G418 +VX-809 +SMG1i		6.2 (4.9% WT)	5.4 (14.7% WT)	4.1

Cmpd concentrations: G418 = 100 mM; VX-809 = 3 µM; SMG1i = 0.3 µM

AUC: Area Under (CFTR Ieq) Curve

WT: same plate control

P54

Bicarbonate directly reduces the mucus micro-viscosity in primary bronchial cells monolayers

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Most of the health problems of cystic fibrosis (CF) patients are caused by the very thick mucus, which is difficult to eliminate. A better hydration of the airway surface liquid (ASL) in not enough to restore a functional mucociliary clearance, but restoration of bicarbonate secretion, and the consequent ASL pH increase, is necessary to correct the mucus phenotype. Treatment of CF cells with lumacaftor partially reduces the mucus viscosity. Thus, we hypothesise that a direct increase of the bicarbonate in the ASL of CF cells would be complementary beneficial in reducing the thickness of the mucus. To investigate the direct role of bicarbonate on airway mucus, we analyze the properties in primary bronchial cells monolayers from normal subjects and CF patients. We applied bicarbonate directly to the apical surface of epithelial models, and the microrheological properties of the ASL were measured from the displacement of fluorescent nanobeads. To test the hypothesis of a direct pH role on the ASL-mucus properties, we have also acidified the ASL by applying lactate on the apical surface of epithelia. We also investigated the role of bicarbonate inhibiting the carbonic anhydrase in the absence of serosal bicarbonate. Our results indicate that the treatment of CF-cells with bicarbonate significantly reduce the viscosity of the mucus. After an incubation of 48h, we observed an increase at least of 20% in the apparent diffusion coefficient of the nanobeads,associated with a reduction of mucus protein concentration in CF epithelia and the hydratation of the ASL. A further viscosity reduction is observed in association with lumacaftor. Experiments confirms the key role of bicarbonate, and pH in general, in determining the mucus thickness. Data obtained here is usefull for the design of strategies for CF treatment patients using inhaled bicarbonate or other "antiacid" compounds. It might represent a mutation-independent and low cost therapy to clear out the mucus that accumulates in the airways, reducing the risk of infections, and improve lung function.

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Analysis of mucus transport mechanisms in mice *in vivo*

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In healthy subjects, mucus is transported in the airways mainly by beating of cilia. Under pathologic conditions such as cystic fibrosis with increased amounts of viscous mucus, additional mechanisms such as airflow and coughing can be necessary to effectively remove mucus. Furthermore, therapeutic interverntions will change mucus properties and possibly also the mechanisms by which mucus is transported. However, at the moment, the mechanism(s) that are operational to remove mucus in vivo cannot be observed directly. To gain insight into mucus transport mechanisms in vivo, we used a custom build microscopic optical coherence tomography (mOCT) setup that allows to visualize the transport of mucus at the ventral side of the intact trachea in anaesthetized spontaneously breathing mice. We studied ß-ENaC overexpressing mice that harbor mucus plugs in their intrapulmonary airways. To mobilize viscous mucus, we applied 30 µl of isotonic saline solution intranasally.

Under basal conditions, we did not detect increased amounts of mucus confirming that the intrapulmonary mucus is too viscous to be spontaneously transported in larger amounts by ciliated cells. In response to intranasal application of isotonic saline, mice responded with increased rapid movements of the thorax. At the same time, we observed that aspirated fluid was rapidly transported back towards the larynx. The speed of the transported fluid was measured to be above 10 mm/s exceeding the speed described for cilia-driven removal of mucus by a factor of 100. After the initial excursion of the thorax, mice acquired a normal breathing pattern. During this period, we observed the cephalic transport of mucus plugs in the trachea with a velocity that was in the range described for cilia-driven transport. Continuous transport lead to accumulation of mucus before the larynx that posed a mechanical barrier for continuous transport. These larger mucus plugs were often transported back and forth in the trachea over several millimeter by breathing. Breathing-mediated transport exceeded the speed described for cilia-driven transport. Despite repeated transport towards the lung by airflow, mucus accumulated before the larynx and led to intermittent blockade of air passage. During this time, we did not detect the initially observed cough-like pattern indicating that blockade of the laryngeal passage is not sufficient to induce this mechanism in anaesthetized mice.

In conclusion, we observed cilia-driven transport, fast transport by rapid exhalation and transport by breathing in vivo. Transmural intravital mOCT imaging of undisturbed mucus transport will be valuable to analyze changes in the primary transport mechanism due to different therapeutic approaches.

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Intramolecular interactions of the airway mucin MUC5B and their role in packaging within secretory granules

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Mucus plays an essential role in forming a barrier to protect the respiratory epithelium from pathogenic and environmental challenges. MUC5B and MUC5AC are the major polymeric, gel-forming mucins in respiratory mucus and are primary determinants of airway mucus properties. MUC5B is stored in the secretory granules of mucous cells, and to a lesser extent in goblet cells, prior to secretion onto the epithelial surface to form mucus. Inside the secretory granule, MUC5B is stored in a dehydrated and compact form; compaction is driven by acidic pH and calcium-rich environment and mediated by calcium-dependent, non-covalent interactions between N-terminal domains of MUC5B (1). After secretion, the increased water availability, neutral pH and lower calcium concentration result in rapid expansion of linear MUC5B polymers via the uncoupling of the interactions between N-terminal domains. The transition from the compact to expanded linear form of MUC5B is vital for the correct rheological properties of the mucus gel and effective mucociliary clearance. In the CF lung, changes in the extracellular milieu (decreased water availability, and increased pH and calcium concentration) contribute to abnormal mucin expansion and conformation, yielding mucus with aberrant transport properties which leads to mucus accumulation and blockage of small airways and recurrent infection (2).

Our objective in this study was to investigate whether other intramolecular interactions between protein domains of MUC5B are active during mucin packaging in secretory granules. Therefore, we have investigated intramolecular interactions between the N- and C-terminal protein domains of MUC5B. The complete N-terminus (D1-D2-D'-D3) and C-terminus (D4-B-C-CK) of MUC5B were expressed in mammalian cells. Recombinant C-terminal MUC5B protein was analysed using multi-angle laser light scattering, analytical centrifugation, small-angle X-ray scattering and transmission electron microscopy. Interactions between N- and C-terminal protein domains were determined using surface plasmon resonance and micro-scale thermophoresis. Structural analysis of the C-terminal dimer of MUC5B identified a rod-like structure, with a flexible region and two globular domains. No homotypic interactions were detected between C-terminal regions. However, results showed that at pH 6.0 in the presence of calcium, the N-terminal and C-terminal of MUC5B formed heterotypic complexes, which were dissociated after calcium chelation by EGTA and increase of pH. These results highlight novel intramolecular interactions between MUC5B protein domains that contribute to the organisation of the mucin molecule during packaging within secretory granules.

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P58

Using *Xenopus tropicalis* to develop a live *in vivo* model of mucociliary clearance

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The deterioration of lung function caused by susceptibility to infection and inflammation are clinical manifestations of cystic fibrosis (CF). Production of thick, sticky mucus that is unable to be moved by normal mucociliary clearance mechanisms creates an environment where pathogens can thrive and overwhelm the body's immune response. A clear molecular understanding of how beating cilia transports mucus and how each of the components of the mucociliary clearance response interact and can be controlled is still lacking. This is due, in part, to the inherent difficulties in studying mucociliary epithelia *in vivo*, in real time. Here we present a new model to study live mucociliary clearance mechanisms - the skin of the *Xenopus tropicalis* tadpole.

As the *X. tropicalis* embryo develops into a tadpole, specialized cells form in the skin to protect the animal from environmental challenges and to exchange gases and nutrients. These cells include motile multi-ciliated cells that beat coordinately and goblet cells that secrete mucin glycoproteins onto the skin surface to form mucus. The tadpole skin is a mucociliary surface with great similarities to the respiratory tract of the human upper airways. But, unlike mammalian research models, it benefits from being open and accessible to the environment. This is particularly advantageous for *in vivo* research including live imaging.

Having very recently identified the major gel-forming mucin, MucXS, which is secreted onto the tadpole skin [1], we have now begun to use CRISPR-Cas9 genome editing at the *mucXS* gene locus for insertion of the sequence coding for the fluorescent protein mEGFP. We successfully targeted *mucXS* to induce a double strand break in the DNA that we repaired using a homology template containing mEGFP. Using an immunofluorescence approach we have shown co-localization of mEGFP with a MucXS antibody in the secretory vesicles. We sequenced the gene to show in-frame integration of mEGFP and are currently raising animals to generate a transgenic line. At the same time, we have also targeted the protein Tau to insert a red fluorescent protein that localizes to the cilia. We are at the stage where an appropriate guide RNA has been identified and are currently constructing the homology template. The aim is to be able to use advanced live imaging approaches to image each of the components together and study how the mucins interact with the cilia. Using this approach, we hope to identify important contributors to mucociliary clearance, mimic disease states and ultimately to translate our findings to human disorders of mucociliary clearance such as in cystic fibrosis.

We have also looked at the expression profile of the cystic fibrosis transmembrane conductance regulator (CFTR) in *X. tropicalis* skin from embryo to adult and suggest the development of a CF frog model to complement mammalian models, specifically to look at the effects on mucin secretion of mimicking mutations in CFTR.

References: 1. Dubaissi E, Rousseau K, Hughes GW, et al (2018) Functional characterization of the mucus barrier on the Xenopus tropicalis skin surface. Proc Natl Acad Sci USA 201713539. doi: 10.1073/pnas.1713539115

P59

Identifying pathways regulating goblet cell metaplasia: phenotypic screening with bronchospheres

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The composition of mucus in the CF airway, and in particular the hydration status, significantly affects its clearance and thereby the potential for form plugs, restrict airflow and create a nidus for chronic microbial colonisation. A variety of largely ion channel-based strategies are being employed to promote mucosal hydration e.g. CFTR repair, ENaC blockers and TMEM16A potentiators. An alternative, but complimentary approach would be to reduce the excessive production and secretion of the mucin proteins that contribute to the solids component of the mucus gel.

One approach to reduce excessive mucus production is to reduce the number of mucus producing goblet cells in the airways. To identify drug targets that could be regulated to achieve this, we have utilised a 3D culture model of the human airway epithelium, 'bronchospheres'. Bronchospheres are derived from primary human airway basal cells, and can be cultured to form a well-differentiated mucociliary epithelium without the need for an air-liquid interface. Bronchospheres are cultured in a 384 well assay format that makes them amenable to medium throughput compound screening. Treating bronchospheres with inflammatory mediators such as IL-13 induces a mucus hypersecretory phenotype with increased numbers of goblet cells and reduced numbers of ciliated cells. Our hypothesis was that the co-administration of test compounds with IL-13 would identify compounds capable of preventing goblet cell formation with the opportunity to seed future drug discovery programs.

Bronchospheres were cultured as previously described (Danahay et al., Cell Rep. (2015) 10(2):239). On day 2 after seeding primary human airway basal cells, treatment with IL-13 ± test compounds was initiated. On day 8, media and treatments were topped-up and on day 14 bronchospheres were lysed and RNA isolated. QPCR was then used to assess the expression of cell-specific markers: MUC5AC (goblet cells) and FOXJ1 (ciliated cells). Compounds that induced a ≥2-fold reduction in expression of MUC5AC were classified as hits. This hit list was then refined by checking the expression of FOXJ1. Compounds that had likewise attenuated FOXJ1 expression by ≥2-fold were deprioritised as these likely represented a non-specific effect on epithelial differentiation. Compounds that either maintained or enhanced FOXJ1 expression in addition to repressing MUC5AC gene expression were prioritised for validation using traditional air-liquid interface cultures.

In total, 92 hit compounds from the bronchosphere screen were tested for effects on goblet and ciliated cell numbers in ALI HBE cultures using quantitative immunohistochemistry. Of these, 38 (41%) significantly attenuated the MUC5AC+ stained area in IL-13 treated HBE and either maintained or increased the FOXJ1+ stained area. Validated hits were then aligned based on their previously reported pharmacological activity to enable common pathways to be identified and to refine our hypotheses through further exemplification of pathway regulators.

Based on this screen, we have identified pathways that regulate the induction and maintenance of a goblet cell metaplasia in vitro and are progressing a lead optimisation program for eventual therapy in respiratory diseases associated with mucus obstruction.

P60
Cholinergic signaling modifies mucus properties

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CF is characterized by stagnant mucus in the airways and chronic infections. Important for the properties of secreted mucins are the conditions at release from the mucin producing cells as shown for MUC2 in the intestine (Ambort D, et al. PNAS. 2012;109:5645-50). Mucus in the ileum is normally easily removable (Ermund A, et al. AJP. 2013;305:G341-7). In contrast, mucus in CF is attached and bacteria are not moved, resulting in intestinal obstruction and bacterial overgrowth (Gustafsson JK, et al. JEM. 2012;209:1263-72) similar to the airways. Bicarbonate via CFTR is essential for formation of normal, easily movable mucus and attached mucus is due to insufficient mucin expansion. The acetylcholine (ACh) analog carbachol (Cch) causes mucus thickness increase in the ileum due to mucus release and expansion. ACh is also a neurotransmitter in the airways (Choi JY, et al. JCI. 2007;117:3118-27). The beneficial effect of anti-cholinergic therapy for chronic obstructive pulmonary disease is well-documented although cholinergic stimulation paradoxically inhibits liquid absorption, increases cilia beat frequency and increases air surface liquid transport. We have now compared mucus expansion and transport in the mouse ileum and pig airways.

Mucociliary transport of Alcian blue stained strands was evaluated by video microscopy of excised distal trachea and primary bronchi from newborn WT and CF pigs mounted in a heated chamber with aerated Krebs-glucose buffer. Mucus attachment was evaluated in intestinal explants from WT and CF mice (Gustafsson JK, et al. JEM. 2012;209:1263-72). In WT and CF mouse ileum, stimulation with the combination Cch and PGE_2 gave the same mucus thickness whereas mucus was not possible to remove in CF, but easily removable in WT. Stimulation with Cch alone gave comparable mucus secretion but thinner mucus in CF compared to WT, the difference being the CFTR-dependent component of mucus expansion. Mucus was also more attached in WT when stimulated with Cch alone compared to the combination, indicating that PGE_2 activated/inhibited additional pathways present in both WT and CF. On pig tracheobronchial explants, the surface liquid transport was increased by Cch. In contrast, the mucus bundles secreted from the submucosal glands were stopped from moving by Cch, an effect counteracted by an anti-muscarinic drug in clinical use. Interestingly, in pigs lacking a functional CFTR channel, the mucin bundles were almost immotile. As in wild-type pigs the CF surface liquid transport increased after stimulation with Cch.

The transport of the surface mucin bundles are dynamically inhibited by ACh and stimulated by the CFTR channel, explaining initiation of CF and COPD and opening novel therapeutic windows.

P61

ENaC has a pivotal role in modulating autoinflammation and glycolysis in cystic fibrosis

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Background: Cystic fibrosis (CF), the most common genetic disorder in European populations, is a multi-system disorder affecting the digestive, endocrine and respiratory systems. The cystic fibrosis transmembrane conductance regulator (CFTR) not only acts as a chloride ion channel but is also an inhibitor of the epithelial sodium channel (ENaC). When absent, both functions are lost resulting in reduced chloride and increased sodium flux. The CF lung is highly inflammatory with elevated innate immune cell infiltrations, associated with high levels of IL-1b. Metabolism and inflammation are inextricably linked, with both systems impacting on each other. This study aimed at understanding the relationship between increased sodium flux with metabolism and NLRP3 inflammasome signalling in CF.

Methods: Human bronchial epithelial cell (HBEC) lines, with various classes of CFTR mutation, were studied. Blood samples were taken from patients with stable CF, active CF, autoinflammatory patients, non-CF bronchiectasis (NCFB) and healthy controls (HC) for monocyte and serum studies. ELISAs were performed for IL-1b, IL-18, IL-1ra, ATP, glucose, succinate and lactate. A Seahorse extracellular flux analyser measured oxygen consumption and proton production. Flow cytometry was used to detect M1-type and M2-type macrophages that were previously polarised *in vitro*, as well as ASC specks. Fluorescent probes for sodium and potassium were used to measure ion flux *in vitro.* Amiloride, lipopolysaccharide (LPS), ATP, 2-Deoxy-D-glucose (2-DG) and MCC950 (NLRP3 inhibitor), were used to treat cells *in vitro*.

Results: Serum levels of IL-1b, IL-18, IL-1Ra and ASC specks were elevated in CF samples compared to HC and patients with NCFB. When monocytes were isolated and polarised to macrophages, there were significantly less anti-inflammatory M2-type macrophages from patients with CF with decreased IL-10 and increased IL-6 secretion. CF monocytes and HBECs had elevated sodium influx upon ATP stimulation and a consequential drop in intracellular potassium levels, a known activator of the NLRP3 inflammasome. Due to the fact that the activity of the sodium/potassium ATP-gated channel is elevated in CF to compensate for increased sodium influx, we measured cellular metabolism to understand how this greater energy demand impacts on glycolysis. ATP, glucose, succinate and lactate were all elevated in CF monocytes and HBECs. Finally, we stimulated monocytes and HBECs with LPS and ATP to activate the NLRP3 inflammasome. We found that monocytes and HBECs from patients with CF hyper-responsive to NLRP3 inflammasome activation and inhibiting ENAC with amiloride reduces this hyper-responsiveness.

Conclusions: Collectively, our findings reveal novel intrinsic mechanisms behind the excessive degrees of inflammation, independent of infection, that are observed in CF. We have shown that IL-1b and IL-18 are dominant in human CF and drive the systemic disease. We demonstrate that CF monocytes and macrophages are skewed towards a proinflammatory phenotype and that increased Na+ influx, via ENaC, contributes to metabolic reprogramming and NLRP3 activation in primary cells from patients with CF and also in cell lines, containing CF-associated mutations. Finally, we show that amiloride, a short-acting small-molecule ENaC inhibitor, is capable of regulating these proinflammatory and metabolic perturbations of the innate immune response in vitro.

P62

Overproduction of IL-6 and TNF through XBP1s is associated with certain CFTR genotypes

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Introduction: The cystic fibrosis transmembrane regulator (CFTR) is a transmembrane protein, involved in the transport of bicarbonate and chloride ions through the cell. CFTR mutations underlie the pathophysiology of cystic fibrosis (CF), including autoinflammation and associated airway infections. The CFTR is assembled and modified in the endoplasmic reticulum (ER) and accumulation of misfolded or unfolded CFTR proteins lead to activation of the unfolded protein response (UPR). The UPR comprises three ER transmembrane proteins, known respectively as PERK, IRE1, and ATF6. UPR activation, through its IRE1 arm, is linked to production of proinflammatory cytokines, including IL-6 and TNF. More than 1900 different CFTR mutations have been identified and classified in seven classes, according to the status of the CFTR protein. We hypothesised that activation of the UPR in CF is present only in certain CFTR genotypes, especially those comprising CFTR accumulation. The objective of this project was to investigate abnormal UPR activation in patients and cell lines harbouring different CFTR mutations.

Methods: Patients' peripheral blood mononuclear cells (PBMCs) and HBEC lines Beas-2b (WT), IB3-1 (ΔF508/W1282X), CuFi-1(ΔF508/ΔF508), CuFi-4(ΔF508/G551D), were used to evaluate the activation of UPR, using quantitative real time PCR (qPCR) and flow-cytometry. IL-6 and TNF production was measured by qPCR and ELISA assays. Tunicamycin (Tn) and thapsigargin (Tg) were used as cellular UPR stimulants to assess UPR activation.

Results: Gene expression revealed a significant increase in *IRE1α, XBP1s, and IL-6* in CuFi-1 and -4 HBEC lines ($P = < 0.05$). IRE1α protein expression was increased in the three CF cell lines; however, phosphorylation of IRE1α was increased only in the CuFi-1 and -4 CF cells. Finally, IL-6 and TNF secretion was significantly increased in all CF cell lines and in patients' PBMCs, and was more pronounced in the CuFi-1 and -4 CF cells.

Conclusion: These data suggest that misfolded CFTR proteins induce IL-6 and TNF thourgh IRE1α activation and XBP1 splicing. This UPR-dependent inflammation is associated with the ΔF508 mutation but, absent in the W1282X CFTR genotype.

P63

Oxidative stress in CF airway epithelium controls cytokine and growth factor shedding

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Current evidence shows that CF lung disease is initiated in CF infants before the onset of bacterial colonization, suggesting an innate CFTR dependent mechanism triggering early inflammation and tissue remodeling. The molecular mechanisms involved are complex and not fully elucidated.

Mass spectrometric analysis of CF infant (age 0-5 years, n=23) BALF showed that lipidomic markers associated with oxidative stress, in particular isoprostanes, correlate with high scores for CF lung disease on chest-CT's (Spearman Rho: 0.060, $P < 0.01$) (Scholte et al in preparation). We hypothesize that this is a CFTR related process in the progression of early lung disease and an important potential therapeutic target. Oxidative stress is at least in part due to activated myeloid cells during acute and chronic inflammation. Here we investigate the contribution of CF airway epithelium to oxidative stress, pro-inflammatory signaling and tissue remodeling, using air-liquid interface cultures of primary bronchial epithelial cells (HBEC-ALI).

First we observed that in CF HBEC-ALI the EGFR/ADAM17 axis, which controls the shedding of multiple growth factors and cytokines involved in chronic lung disease [Stolarczyk et al, 2018 Mediators of Inflamm doi:10.1155/2018/1067134], is more active than normal and substantially reduced by supplementing the medium with glutathione (GSH) [Stolarczyk et al 2018 AJP, in press]. Since ADAM17 is activated by oxidation of the extracellular proteolytic domain involved in shedding activity, this is consistent with a more oxidized extracellular milieu in CF epithelia, likely due to deficient GSH transport. Indeed, we observe enhanced markers of oxidative stress (malonyldialdehyde and nitroTyrosine) in CF HBEC compared to WT, which is virtually abolished by GSH treatment. Similar results were obtained using BEC-ALI cultures generated from CFTR KO pig compared to littermate controls (Nick Klymiuc et al, Muenchen) validating of our findings in an independent system.

Analysis of CF HBEC-ALI conditioned basal media using a Fluidigm based 96x96 protein array (Olink™) revealed that basal shedding of several signalling molecules involved in neutrophil and macrophage migration and chemotaxis (CX3CL1, CCL20, CXCL5, CXCL1,IL8) and cell proliferation and angiogenesis (CX3CL1, TNFRSF12A, VEGFA, CXCL10, IL8) is reduced by GSH supplementation (>2-fold, $P< 0.01$).

Together, these data show that unchallenged CFTR deficient epithelia in ALI culture are characterised by significant increase in the expression of oxidative stress markers, likely related to deficient GSH transport. This is associated with enhanced shedding of growth factors and pro-inflammatory signalling proteins. Since we observe oxidative stress markers in CF infant BALF that correlate with CF lung disease, we propose that this mechanism plays a role in the initiation and progression of early CF lung disease. Early intervention targeting these pathways may be beneficial to CF patients.

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P65

CFTR correction by lumacaftor+ivacaftor combo does not diminish the rhinovirus- or interleukin-β -stimulated interleukin-8 in primary cystic fibrosis bronchial epithelial cells

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Rationale: Upper airway infections by Human Rhinovirus (HRV) often precipitate exacerbations of Cystic Fibrosis (CF) lung disease. CF bronchial epithelial cells expressing the delF508 CFTR mutation show a delayed HRV clearance *in vitro* (Dauletbaev N et al. 2015; Schögler A et al. 2015). HRV stimulates inflammatory response from airway epithelium. HRV persistence and associated inflammatory responses may aggravate chronic airway inflammation in CF, contributing to exacerbations.

Research Question: We asked whether HRV-stimulated inflammatory response could be beneficially modulated in bronchial epithelial cells via CFTR correction by the clinical drug ORKAMBI.

Methods: We cultured submerged primary CF bronchial epithelial cells until approximately 80-90% confluency. Afterwards, the cells were treated for 24 hours with diluent or combinations of lumacaftor+ivacaftor (to recapitulate ORKAMBI; respective doses of 500 / 10 nM or 1000 / 100 nM). CFTR correction was judged by Western blot analysis of CFTR band C expression. In some experiments, cells were differentiated for 4+ weeks on Transwells, treated basolaterally for 24 hours with lumacaftor+ivacaftor, and subjected to Ussing chamber assay to document CFTR correction. Following treatment, submerged cells were inoculated for 2 hours with HRV16 at the Multiplicity of Infection of 0.1. Thereafter, cells were washed three times to remove unattached virus, and supplemented with fresh diluent or lumacaftor+ivacaftor. At 22 hours post-inoculation, supernatants were collected for Interleukin-8 ELISA to assess the cell inflammatory response. As a positive control for Interleukin-8 upregulation, we stimulated parallel cells for 24 hours with 10 ng/ml Interleukin-1β (with or without pre-treatment with diluent or lumacaftor+ivacaftor).

Results: Pharmacological correction of CFTR could not be detected by Western blot analysis of CFTR band C expression in submerged cells, but was documented by the Ussing chamber assay in well-differentiated cells. In submerged cells, inoculation with HRV16 led to a 3.3-fold upregulation of Interleukin-8 (*vs* 6.8-fold upregulation by Interleukin-1β). Neither of the tested lumacaftor+ivacaftor concentrations decreased the upregulation of Interleukin-8 stimulated by HRV16 or Interleukin-1β. **Discussion**: In submerged primary CF bronchial epithelial cells, lumacaftor+ivacaftor treatment (i.e., ORKAMBI) does not substantially diminish the upregulation of Interleukin-8 stimulated by HRV or inflammatory stimuli. Studies with well-differentiated primary CF bronchial epithelial cells are underway to confirm the results from submerged cells.

- ND and MT are co-first authors.

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P66

L iminosugars: new anti-inflammatory drugs for CF lung disease?

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Novel drugs tailored to CF lung pathology able to reduce the tissue damage due to the excessive inflammation in CF patients are still an unmet need. Protective inflammatory response during airway infection is mediated by sphingolipids (SLs) (Sharma L and Prakash H, 2017), therefore SL metabolism could be modified to benefit host defense. In this regard, several inhibitors have been proposed. The iminosugar N-butyl d-deoxynojirimycin (d-NBDNJ, Miglustat) produces an anti-inflammatory effect in CF bronchial cells, by targeting β-glucosidase 2 (GBA2) (Loberto N, 2014). Iminosugars are attractive carbohydrate mimics and represent lead compounds for developing new drugs for many diseases (Horne G, 2011). The high potential of l-iminosugars as novel drug candidates has been recently proposed (D'Alonzo, 2017). Interestingly, l-iminosugars have demonstrated to be able to efficiently recognize and inhibit d-glycosidases (Horne, 2011). To better exploit the benefits of iminosugars, we focused on l-enantiomers and on racemic mixtures composed by d- and l-enantiomers. In order to identify similar chemical compounds able to reduce the inflammatory response to *P.aeruginosa* at low dosage, the effects of these compounds in CF bronchial cells and murine models of lung infection were studied. Nanomolar concentrations of monovalent and multivalent DNJ iminosugars and racemic mixtures of N-alkylated iminosugars produced an anti-inflammatory effect in CF bronchial cells. Combination of low doses of miglustat and its l-enantiomer was more potent than miglustat alone and reduced the inflammatory response to *P.aeruginosa* also in CF primary cells. Analysis of d- and l-iminosugars demonstrated that l-enantiomers were more specific than the corresponding d-isomer for GBA2. No toxicity was observed both *in vitro* and *in vivo*. Combination of low doses (10 mg/Kg) of miglustat and its l-enantiomer administered to C57Bl/6NCr mice 24 hours before bacterial challenge improved the resistance to infection. Very importantly, treatment with l- but not d-enantiomer alone significantly reduced the amount of neutrophils recruited in bronchoalveolar lavage without increasing the bacterial load. These results confirm that DNJ-containing iminosugars are effective in reducing the inflammatory response to *P.aeruginosa* in CF bronchial cells. Our novel findings *in vivo* give a new insight into therapeutic approaches for managing respiratory infections by using N-alkylated l-iminosugars that can be regarded to improve effectiveness even at low dosage and to address selectively more specific targets.

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P67

Normalising inflammation in CF airways by DREAM inhibitors

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Airway epithelial cells in Cystic Fibrosis (CF) have a pro-inflammatory phenotype facilitated in part by a lack of A20 (Kelly C *et al.* Eur Respir J 2013) contributing to persistent NF-kB activation. A20 transcription is regulated by DREAM, the **D**ownstream **R**egulatory **E**lement **A**ntagonist **M**odulator, which functions as a transcriptional repressor of A20) and CF airway epithelial cells (CFBEo- and primary nasal epithelial cells) show increased mRNA and nuclear expression of DREAM.

We have previously used an advanced bioinformatics approach (connectivity mapping) to predict drugs already licensed for the use in humans able to induce A20 and thereby reducing the inflammatory response (Malcomson B. *et al* PNAS 2017). We have now used SscMap to predict small compounds able to reduce DREAM mRNA. Here we investigate the effect DREAM inhibitors on the LPS-induced inflammatory response.

Hypothesis: In CF, reducing excessive DREAM expression can normalize inflammation through induction of the NF-kB regulator A20.

Methods: We used bronchial epithelial cells (CFBE41o- and non-CF bronchial cells)

stimulated with LPS from *P. aeruginosa* (10 mg/ml, 0-24h) in the presence of predicted DREAM inhibitors (SscMAP) and determined IL-8 release in cell free supernatant and DREAM mRNA expression using ELISA and qRT-PCR respectively.

Results: Bambuterol was predicted to reduce DREAM mRNA in CF airway epithelial cells. While bambuterol did not have any significant anti-inflammatory effect on control airway cells, bambuterol significantly reduced IL-8 release in CFBE41o- cells (0.1 -100 µM).

Conclusion: Reducing the excessive DREAM expression in CF airway epithelial cells normalizes the inflammatory response of the airways to LPS, providing alternative anti-inflammatory treatments. Drug repositioning is able to provide CF patients with such anti-inflammatory treatments faster than conventional drug development.

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Impact of F508del mutation on lipoxygenases expression and localisation in Cystic Fibrosis

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Background and aim: Chronic lung infection and inflammation is one of the major mechanism responsible for the airway damage of patients with Cystic Fibrosis (CF) and is classically explained by the airway dehydration due to CFTR mutation and altered mucociliary clearance of bacteria. In addition an abnormal production of the pro-resolving lipid mediators such as Lipoxin A4 (LXA4) and Resovlin D1 (RvD1) has been reported in airway of CF patients but the cellular and molecular mechanism involved are not known. During the inflammatory process the balance between pro-inflammatory (Leucotrienne B4) and anti-inflammatory (LXA4, RvD1) molecules is tightly regulated by the expression and activation of several enzymes involved is the lipid metabolism including: 5-lipoxygenase (5-LO), 15-lipoxygenase (15-LO), 12-lipoxygenase (12-LO), leukotriene hydrolase (LTA4H) and cyclooxygenase-2 (COX-2). The aim of the study was to characterize the impact of CFTR mutation on these enzymes expression and localisation in human airway epithelial cells.

Methods: Using RT-qPCR, Western blot and immunofluorescence confocal microscopy analysis we compared the expression levels and localisation of these enzymes on airway epithelial cells expressing WT-CFTR (WT CFBE) or F508del-CFTR (F508del CFBE) under air/liquid interface.

Results: Our results provide evidence for 15-LO1, 15-LO2, 12-LO, 5-LO, LTA4H and COX-2 expression in both WT CFBE and F508del CFBE cells. 15-LO2 expression decreased in F508del CFBE. The 5-LO detection increased at the nuclear membrane level of F508del CFBE while this enzyme appeared to be diffused in the cytoplasm of WT CFBE. LXA4 (10nM) or RvD1 (10nM) treatment decreased 5-LO localisation at the nuclear membrane.

Conclusion: These results showed for the first time that 5-LO is expressed in bronchial epithelial cells and not only in neutrophils and macrophages. The 5-LO localisation at the nuclear membrane and the decrease level of 15-LO2 are consistent with an increased biosynthesis of LTB4 at the expense of LXA4 in CFBE expressing F508del CFTR. The down regulating effect of LXA4 and RvD1 suggest that these SPM could correct their defective production in CF airways.

P69

Longitudinal analysis of the airways' microbiota in patient with Cystic Fibrosis

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In patients with cystic fibrosis, several next generation sequencing based studies were established in the last decade to decipher cross-sectionally the structure and composition of the airways' microbiota. The aim of our study was to analyze the evolution of the microbiota among the airways (nose, throat and lung).

Using 16S amplicon sequencing, we analyzed 87 nose swabs from 10 patients, 462 throat swabs from 43 patients, and 763 sputum samples from 60 patients for an average time period of 3 years.

We observed that each patient possessed a personalized microbiomeas the Morisita-Horn distance between patients' related microbiomes was lower than between non-related microbiomes for the three airways' compartmenst. Our results also showed that the establishment of a chronic infection by *P. aeruginosa* was increasing the instability of the throat microbiome while it was decreasing the instability in the sputum indicating that the establishment of the infection in the lower airway correlates with a dysbiosis in the upper area. *P. aeruginosa* infection in the lower airway was also correlated to an accelerated decline of the alpha-diversity per year as well as *Staphylococcus* infection. In contrast, anaerobes(*Prevotella* and *Veillonella*) were correlated with a stabilization of the decline in the alpha-diversity per year. Finally, we observed that dominance was correlated to an increase of the stability of the microbiome over the time indicating a failure in the elimination process by the lung.

In conclusion, our results show that the evolution of the microbiome in the lower airway is mostly dependent of the acquisition of a chronic infection by a CF pathogen which is correlated with a break in the dynamic colonization/elimination of the lower airways. On the other side, the maintenance of anaerobes in the lower airways was correlated to a more dynamic microbiome with a stable/increased alpha-diversity over the years.

P70

Airway surface liquid acidification initiates host defense abnormalities in Cystic Fibrosis

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Background: Cystic fibrosis (CF) is a lethal autosomal recessive genetic disorder caused by mutations in the *CFTR* (Cystic Fibrosis Transmembrane conductance Regulator) gene encoding the CFTR protein, an anionic channel, secreting chloride (Cl^-) and bicarbonate (HCO_3^-). Most of the patients are homozygous for the F508del mutation. The major cause of morbidity is infection of the airways which starts from the first hours of life, mainly involving *Staphylococcus aureus*. Data in the CF pig model established a relationship between an acidic airway surface liquid (ASL) and a defective antimicrobial capacity. Studies in CF patients are lacking to clearly understand the initial mechanisms that impair bacterial clearance.

Objectives: To investigate how the defect in CFTR function impacts on ASL pH and its relationship with antimicrobial capacity in human airways.

Methods: Real-time measurement of ASL pH from polarized cultures of human Cystic Fibrosis Bronchial Epithelial cell lines (CFBE 41o$^-$) cells and primary cells from healthy controls and F508del homozygous patients was investigated with a pH microelectrode in physiological (25 mM HCO_3^-, 5% CO_2) and extracellular normocapnic acidosis (10 mM HCO_3^-, 5% CO_2) conditions, upon pharmacological modulation of transporters of interest.

Bicarbonate secretion was assessed by short circuit current (SCC) in Ussing chamber upon mucosal-to-serosal HCO_3^- gradient across the epithelium in low Cl^- solutions.

Antimicrobial capacity of the airway cell cultures was studied after *S. aureus* CIP 76.25 (Collection de l'Institut Pasteur) apical infection by counting survival bacteria Colony Forming Unit at different time points.

The activity of the cathelicidin-related peptide, LL-37 and the hBD1 defensin were checked at different pH conditions.

Results: In both CFBE cell lines and primary cells, ASL pH was significantly lower in CF than in Wild Type (WT) CFBE 41o$^-$ cells and bronchial primary cells. CF cell lines and primary cells demonstrated a defect in ASL pH adaptation upon extracellular normocapnic acidosis. This was consistent with a defect in cAMP dependent HCO_3^- secretion by the CF epithelium, as assessed by SCC experiments. The anion exchanger SLC26A4 (pendrin), the non-gastric H^+/K^+ adenosine triphosphate (ATP12A) and CFTR at the apical side at the apical side contribute to pH homeostasis in human airways.

CF ASL demonstrated a defect in antimicrobial capacity which was improved by normalization of ASL pH in CF airways and related to a pH dependent LL-37 and hBD1 activities. Pendrin inhibition in WT airways recapitulated CF airway defect as assessed by acidified ASL and increased bacterial proliferation.

Conclusion: ASL pH impacts on its bacterial clearance capacity and the activity of the antimicrobial peptides. Increasing ASL pH might be one of the factors to improve innate airway defense in newborns with CF. Pendrin activation could represent a major therapeutic target.

P71

Lack of CFTR results in impaired innate immunity in small airways

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It is often assumed that cystic fibrosis (CF) lung disease begins in the small airways. While a number of observations suggest this assumption is correct, we do not have direct experimental evidence. Because we have more knowledge of host defense defects in large CF airways, we might conjecture that the abnormalities in small CF airways are the same. However, differences in epithelial morphology, cell types and gene expression profile, and lack of submucosal glands and continuous cartilages suggest that it is not safe to assume that small airways are simply "small" large airways. Our preliminary data show that newborn CF small airways are more acidic than non-CF, and have an impairment in host defenses. Bacterial killing by airway surface liquid is impaired compared to non-CF, and mucociliary transport is altered in CF small airways after stimulation of goblet cell secretion with ATP. In large airways CFTR and ATP12A regulate ASL pH and innate immunity. We found that small airways do not express ATP12A, instead they seem to rely on V-ATPase for H^+ secretion. We found that V-ATPase is expressed on the apical surface of a specific cell type of small airway epithelial cells. An isoform of the ATP6V0D subunit, ATP6V0D2, is required for apical localization of V-ATPase in the small airways, and interventions can regulate the translocation of V-ATPase to the apical membrane. Understanding the contribution of small airways to disease and in identifying strategies for better treatments/cures of CF. It will also educate us about small airways physiology and how it may be abnormal in other diseases.

P72

Regulation of cathelicidin LL-37 and cyclooxygenase expression by *Pseudomonas aeruginosa* in human bronchial Cystic Fibrosis epithelial cells

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Introduction: In the lung, mutations of CFTR cause depletion of airway surface liquid and mucus dehydration, leading to an appropriate niche for bacterial infection and inflammation. Cathelicidin LL-37 is an antimicrobial peptide (AMP) expressed as a propeptide, called CAP-18, produced by airways epithelial cells (AECs) and cleaved on its C-terminal domain to release LL-37. Cyclooxygenases (COXs) enzymes are known to produce prostaglandins (including PGE2) mediators that play a role in CF airways inflammation.

Aim: the major aim of this work was to examine the modulation by *Pseudomonas aeruginosa* infection of the cathelicidin CAP-18 and COX expression and identify the bacterial determinants and host signaling pathways involved in these expressions.

Methods: The CF-derived bronchial epithelial IB3-1 cell lines were infected by the wt laboratory strain of *P. aeruginosa* (PAK) and its mutants at an MOI of 1 and 5. Expression of CAP-18 and COX-2 (the inducible form of COXs) was examined, 24- and 48-hrs post-infection, at mRNA and protein levels by RT-qPCR and western blot, respectively. In addition, PGE2 levels were determined by ELISA in cell supernatants.

Results: Our results showed that the wt PAK strain decreased mRNA and protein expression of CAP-18 in IB3-1 cells line. Interestingly, the PAK mutant ΔpscF, lacking the type-3 secretion system (T3SS, known to inject bacterial exotoxins in host cells), up-regulated CAP-18 expression compared to the wt PAK strain. Besides, we also showed that the PAK mutant ΔExoS, lacking the exotoxin ExoS, increased CAP-18 expression at both mRNA and protein levels. However, other PAK mutants such as ΔExoT and ΔExoY (lacking the exotoxin T and Y, respectively) had no effects on CAP-18 expression. Our studies also showed that NF-kB, AP-1, SP1, p38 and Erk-MAPKs are not involved in ExoS-mediated down-regulation of CAP-18. Finally, we showed that the wt PAK increased COX-2 expression and PGE2 release in IB3 cells, compared to the ΔExoS mutant that was unable to stimulate these processes. Previous data in literature have shown that COX-2 metabolites play a role in the modulation of CAP-18 expression in non-CF cells. This role is now under investigation in our cell system IB3 cells.

P73

Pseudomonas aeruginosa LasB subverts innate immunity in alveolar macrophages and lung epithelial cells

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Introduction: *Pseudomonas aeruginosa* (*P.a*) is an important pathogen in cystic fibrosis. The *P.a* T2SS LasB is a major virulence factor that targets lung epithelial-derived IL-6 and the antimicrobial molecule trappin-2 (T2, (Saint-Criq et al, Thorax, 2017)). We have also shown, using a transgenic approach, that *in vivo* over-expression of these molecules can rescue that deleterious phenotype. Although this can be partly explained by an effect of LasB on lung epithelial cells, we also considered here the role of the alveolar macrophage, a cell type which had seldomly been considered previously in that context.

Aims: 1) Investigate how LasB can impair alveolar macrophages (AM) and epithelial cells (ECs) production of cytokines & antimicrobial molecules/AMMs (eg Trappin-2/T2, Lcn2, S100a8, S100a9..).

2) Test whether Adenovirus (Ad)-mediated over-expression of IL-6 and/or T2 can modulate epithelial cellular responses and amplify the rescuing and protective phenotype.

3) Analyse specifically the contribution of AMs in the protection against LasB-mediated bacterial infection and lung inflammation.

Materials & Methods: A) After treatment during 4hrs with WT PAO1 or ΔLasB secretomes (SEC, 1%), we measured IL-6, IL-1ß and IL-23 levels (q-PCR and ELISA) in the AM MPI cell line (cell lysates and supernatants), and those of AMMs in the CMT alveolar and the DJS Club Cell line.

B) Using MPI as the model AM cell line, cells were either 'Mock-treated' (MPI control, n=2), or infected with WT- or ΔLasB-PAO1 (moi 1, n=3)) during 4hrs. Supernatant was then recovered, concentrated, and analysed for LC-MS/MS (mass spectrometry) acquisition.

Results: 1) We showed *in vitro* that compared to ΔLasB-SEC, WT-SEC decreased MPI-derived IL-6 & IL-23 levels by a factor of 7,2 & 20, respectively. In CMT, but not DJS Cells, WT-SEC down regulated Lcn2, S100a8, S100a9 mRNA levels by a factor of 3,1, 4,5 & 5 respectively.

2) In MPI cells, Ad-IL-6 treatment up-regulated S100A8 & Lcn2 (1.5 & 9 fold respectively) whereas Ad-T2 increased S100A8, S100A9& Lcn2 3.5, 15 & 9 fold respectively. In DJS cells, Ad-IL-6 & Ad-T2 up-regulated the AMP CAMP 10 and 2 fold, respectively.

3) Proteomics analysis : when compared side by side, the condition WTp0-PAO1>ΔLasBp0-PAO1 (fold-increase>2, $p< 0.05$) identified higher levels of 203 proteins whereas that of ΔLasBp0-PAO1 >WTp0-PAO1 (fold-increase>2, $p< 0.05$) identified higher levels of only 40 proteins. For the latter comparison, proteins involved in innate immune responses were particularly present (Il-2rg, H2-D1, IL12b, Ifnar2, complement factor B, complement C3, Sepp1, Chill3, Chill4, macrophage Csf1r, cathepsin-B, cathepsin-H, cystatin). Interestingly, most of these proteins were also found up-regulated in the MPI control >WT-PAO1 + MPI condition, suggesting altogether that these mediators are likely important innate immune targets for LasB-mediated subversive strategies.

Discussion: We show here that both ECs and AMs are important targets for the *P.a* LasB virulence factor and identify key protective targets secreted by AM. Furthermore, we show that over-epressing IL-6 and T2 may hamper the early onset of *Pseudomonas aeruginosa* within the alveolar lumen in susceptible individuals, such as cystic fibrosis patients, where a deficit in phagocytic/killing activity in alveolar macrophages has been implicated.

P74

The Cif virulence factor perturbs airway defenses and exacerbates lung damage

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The opportunistic pathogen *Pseudomonas aeruginosa* (Pa) infects and colonizes the lungs of vulnerable patients, including most adult patients with cystic fibrosis (CF). In doing so, it exploits multiple systems to survive and even thrive in a hostile inflammatory environment. Many of these systems are defensive, including biofilm formation, metabolic tuning, and nutrient scavenging. Others actively subvert both host immune responses and treatments to correct the basic defect in CF: loss of function of the cystic fibrosis transmembrane conductance regulator (CFTR). In particular, Pa secretes the CFTR inhibitory factor (Cif), an epoxide hydrolase (EH) that triggers degradation of CFTR from the apical membrane of airway epithelial cells. It also directly counteracts the ability of modulators to rescue the most common disease-associated mutant protein (F508del-CFTR). Here we present a robust system for analyzing mucociliary transport across reconstituted airway epithelia, and show that Cif rapidly inhibits mucociliary transport. Furthermore, Cif hydrolyzes an epoxy-fatty acid in a signaling pathway required to help resolve inflammatory responses. As a result, Cif can promote a hyper-inflammatory environment that is beneficial to the microbe and damaging to the host. Cif is expressed in clinical isolates and persists in longitudinal samples from chronically infected patients for up to 15 years. In bronchoalveolar lavage fluid, higher levels of Cif correlate with higher levels of inflammatory signals, lower levels of the resolving signal 15-epi lipoxin A_4, and worse airway function. We have now adapted a mouse pneumonia model to test the multiple virulence effects of Cif. We have confirmed that Pa expressing WT Cif is able to remain in the airway at higher levels than Pa strains genetically engineered to express an enzymatically inactive form of Cif. In addition, although both Pa strains induce similar levels of neutrophil recruitment, levels of 15-epi lipoxin A_4 in response to infection by the Cif-mutant strain are higher than levels seen for Cif-WT, confirming the ability of Cif to interfere with pro-resolving pathways. Together, our results establish that Cif is a bona fide virulence factor. It sabotages important immune regulatory mechanisms and thus promotes damage to patient airways. Finally, leveraging our understanding of Cif's active site chemistry, we have used biochemical assays and X-ray crystallography to develop Cif inhibitors with sub-micromolar affinity. Together with our in vivo and reconstituted systems, these tools enable us to refine our understanding of Cif as a therapeutic target and as a mediator of novel host-pathogen interactions.

P75

Protease deficient *Pseudomonas aeruginosa* isolates are common in cystic fibrosis infections and cause exaggerated pulmonary inflammation

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Chronic *Pseudomonas aeruginosa* (*Pa*) airway infections are persistent and associated with severe neutrophilic inflammation. *Pa* produces several secreted proteases, including LasB (*Pa* elastase), and nearly two thirds of CF-adapted *Pa* clinical isolates are protease deficient. Our group has recently reported that *Pa lasR* variants are impaired in secreted elastase activity and are associated with hyper-inflammatory responses in airway epithelial cell cultures and murine subacute lung infections. We now hypothesize that LasB, the major Pa secreted elastase, dampens innate immune and inflammatory responses, and that the loss of secreted elastase in CF-adapted *Pa* is sufficient to cause increased pulmonary immunopathology.

We characterized a collection of 167 CF *Pa* clinical isolates for secreted protease activity and sequenced their *lasR* gene. Among these isolates, 42 (25%) are protease deficient, and all protease deficient isolates carry LasR loss of function mutations. Furthermore, we tested 18 pairs of clonally related CF clinical isolates (one early and one chronic stage infection) and found that 67% of late stage (CF-adapted) Pa isolates are elastase deficient compared to their early stage ancestral isolate. Although Pa populations are likely heterogeneous in CF sputum, protease-deficient variants are predominant when present. Since LasB potently degrades many host proteins, including inflammatory cytokines and growth factors, we sought to investigate the contribution of LasB in modulating host innate immune and inflammatory response. We constructed a *lasB* deletion mutant (*ΔlasB*) in a CF clinical isolate. Using an in-vitro model of immortalized airway epithelial cell (AEC) cultures (Beas-2B and CFBE), we observed that stimulation of AEC with the *ΔlasB* mutant was associated with higher levels of secreted pro-inflammatory cytokines and growth factors, including IL-6, IL-8, G-CSF and GM-CSF compared to its wild-type parental isolate (WT). We also show that *ΔlasB* endobronchial lung infection, using a murine model of chronic airway infection with *Pa* embedded in agar beads, causes greater pulmonary neutrophilia and immunopathology, and is associated with higher pulmonary and systemic pro-inflammatory cytokine response (IL-6, KC, MCP-1, G-CSF, TNF-a).

Our results suggest that elastase deficient mutants are common in CF patients and are caused by *lasR* mutations. Loss of secreted elastase is sufficient to causes exaggerated host inflammation *in vitro* and *in vivo.* These results suggest the loss of secreted elastase in CF adapted *Pa* isolates modulates host inflammatory responses and may contribute to the progression of lung disease in CF patients.

P76

Effect of anaerobiosis on virulence of *Pseudomonas aeruginosa*

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P. aeruginosa is adept at establishing a lifelong infection in the lung of cystic fibrosis (CF) patients, which is not resolved by antibiotic treatment and the patient's immune response. Chronic infection with *P. aeruginosa* is associated with a rapid decline in lung function. Accumulating evidence indicates that hypoxic and anoxic niches are common in the lung of CF patients including pediatric patients. However, the influence of oxygen limitation on virulence traits of *P. aeruginosa* in particular on the secretome is poorly understood. In this study, we investigated the effect of secreted compounds of *P. aeruginosa* incubated under aerobic and anaerobic conditions on the viability of wax moth larvae and mammalian cells in cell culture. To this end, the bacteria were grown in M9 minimal medium in a fermenter under defined aerobic and anaerobic conditions. Incubation was performed with constant stirring to prevent surface contact and biofilm formation of the bacteria. When the OD_{600nm} reached 1.0 (exponential phase), cell free culture supernatants were prepared and added to lung epithelial cells (A549) and macrophages (RAW264.7). The protein concentration of the supernatants was similar between the treatments ($+O_2$: 260 µg/ml; $-O_2$: 270 µg/ml). At 3 h post incubation cellular viability was determined by measuring esterase activity and plasma membrane integrity at the single cell level using a fluorescence based assay (N > 650 cells/treatment). We found that culture supernatants prepared from *P. aeruginosa* grown under anaerobic conditions were significantly more toxic upon A549 cells as compared with bacterial supernatants of aerobic cultures (20% vs. 40% cell death). Likewise, the number of dead RAW264.7 macrophages was significantly higher after exposure to anaerobic supernatants relative to aerobic supernatants (26% vs. 50%) indicating that oxygen deprivation stimulates the secretion of toxic compounds. Both boiling and Proteinase K treatment of the supernatants abolished the difference between the toxicity of aerobic and anaerobic supernatants upon A549 cells. This result suggested that proteins in the anaerobic bacterial supernatant contribute to cell death. Furthermore, *Galleria mellonella* larvae (TruLarv) were used as a model to analyze the toxicity of supernatants *in vivo* (N > 24 larvae/treatment). Consistent with the findings obtained *in vitro* we observed rapid killing of the larvae upon hemocoel injection with anaerobic supernatants but not with aerobic supernatants: 100% of the larvae injected with anaerobic supernatants were killed within 24 h whereas 15% of the larvae challenged with aerobic supernatant died within 24 h and 56.7% survived until the end of the monitoring period. Boiling of the supernatants abrogated the toxic activity and resulted in 100% larval survival. A detailed analysis of the composition of the anaerobic supernatants is ongoing to identify hitherto unknown virulence factors of *P. aeruginosa* that facilitate long term survival in the CF lung.

P77

Intracellular persistence of *Pseudomonas aeruginosa* in airway epithelial cells and CF lungs

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Cystic Fibrosis (CF) lung disease is characterized by chronic *Pseudomonas aeruginosa* infections in the CF airways. Why *P. aeruginosa* persists despite antimicrobial treatments and activation of innate immune responses remains incompletely understood. To date, the prevailing view suggests that impaired mucocilliary clearance and *P. aeruginosa*'s biofilm lifestyle are the primary mechanisms contributing to the persistence and failed eradication of *P. aeruginosa* in the CF lung. Although *P. aeruginosa* is primarily known as an extracellular pathogen, previous studies have observed that *P. aeruginosa*'s can be internalized by epithelial cells *in vitro*. What the biological relevance of this process and whether it occurs in the CF lung remains largely unknown. This led us to hypothesize that *P. aeruginosa* can be internalized and persist in airway epithelial cells, and that this unrecognized intracellular lifestyle contributes to *P. aeruginosa*'s evasion of extracellular antibacterial drugs and host defenses.

We have developed a novel *in vitro* model of long-term intracellular persistence of *P. aeruginosa* in human airway epithelial cells using several cell lines (Beas-2B, CBFE-WT and CFBEΔF508), and have tested different *P. aeruginosa* strains, including the reference strain PAO1, mucoid and non- mucoid CF clinical isolates, and several genetically engineered mutants deficient in T3SS, flagellar motility among others. We observed that *P. aeruginosa* persists and replicates in B2B and CFBE cells over 5 days without activating intracellular inflammatory signaling pathways, or causing significant host cell cytotoxicity and death. Approximately 5 to 10% of the initial bacterial inoculum is internalized, and the intracellular bacterial burden increases 2-fold over 5 days. Moreover, intracellular *P. aeruginosa* is increased ~ 2-fold in CFBEΔF508 cells compared to CFBE-WT cells, and this is reversed with VX-770/VX-809. Loss of flagellin promotes *P. aeruginosa* persistence in airway epithelial cells, while no difference is observed between T3SS(+) and T3SS(-) strains.

In order to determine whether the CF lung harbor intracellular *P. aeruginosa*, we examined lung tissues from CF lung explants. Preliminary semi-quantitative microbiological analyses and 3D confocal microscopy imaging by MiPACT (Microbial Identification Passive CLARITY Technique) coupled with in situ localization of *P. aeruginosa* within lung tissues by immunofluorescence and hybridization chain reaction, suggest the presence of intracellular *P. aeruginosa* in lung epithelial cells.

In summary, our *in-vitro* data suggests that *P. aeruginosa* can be internalized and persists intracellularly within human airway epithelial cell cultures. Preliminary results also suggest that CF lung explants harbour intracellular *P. aeruginosa* within lung epithelial cells. These findings raise the possibility that *P. aeruginosa* may have an unrecognized intracellular reservoir within the CF lung and highlight a potentially new mechanism of bacterial persistence.

P78

Inflammatory response of respiratory epithelial cells to *Aspergillus fumigatus*: a role of the Syk pathway in absence of Dectin-1 involvement

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Rationale:*Aspergillus fumigatus* (*A.f*) is an opportunistic fungus frequently recovered in the sputum of cystic fibrosis (CF) and *chronic obstructive pulmonary disease* (COPD) patients. Inhalation of spores results in fungal growth inside the lung and hematogenous dissemination. Although it is generally accepted that alveolar macrophages kill conidia while neutrophils lyse hyphae, we previously demonstrated (Balloy *et al*., 2008) that respiratory epithelial cells (REC) sense germinated spores of *A.f* and thereby be activated at least through two different pathways: i) a MyD88 independent one leading to IL-8 synthesis *via* PI3 kinase, p38 MAPK, ERK1/2, AP-1 and NF-kB activation, and ii) a MyD88 dependent one involving NF-kB activation too. Here, we studied in more details those two pathways.

Methods: The human bronchial epithelial cell line BEAS-2B was infected with *A.f* conidia. Cytokine concentrations in cell culture medium were determined by ELISA or multiplex analysis. To assess the role of the different pathways, target proteins were knockdown using specific siRNA or their biological activities dampened with pharmacological inhibitors. siRNA efficiency was controlled by measuring target mRNA expression by Taqman PCR.

Results: Through a multiplex analysis of 27 inflammatory mediators, we observed that only IL-8 and IL-6 were significantly synthesized by *A.f*-stimulated REC and this independently of the MyD88 pathway.

We then looked for the involvement of Syk, a immunoregulatory protein tyrosine kinase involved in most antifungal responses. We observed that Syk was required (siRNA and piceatanol) for *A.f* -induced PI3 kinase, p38 MAPK, ERK1/2, NF-kB activation and cytokines synthesis. Syk activation is often associated with C-type lectin-like receptors, such as Dectin-1 involved in the recognition of *A.f* b-glucan by alveolar macrophages. We observed that Dectin-1 was expressed at a very low level in REC activated or not by *A.f*, and that its knockdown did not modify cytokine synthesis triggered by *A.f*.

Conclusions: The study demonstrates that Syk mediates kinases and NF-kB activations and cytokine synthesis in *A.f* -stimulated REC but independently of the C-type lectin-like receptor Dectin-1.

Future studies will look for which receptor is involved in the recognition of *A.f* and cytokine synthesis. As filamentous fungi may contribute to the local inflammatory response, and therefore to the progressive deterioration of the lung function in CF and COPD, results could provide potential targets for designing therapeutic agents against *A.f* infection.

P79

CFTR correction by lumacaftor+ivacaftor combo does not diminish rhinovirus RNA titer in primary cystic fibrosis bronchial epithelial cells

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Rationale: Lung disease in Cystic Fibrosis (CF) is frequently exacerbated following acute upper respiratory virus infections, most commonly caused by the Human Rhinovirus (HRV). We and others showed that HRV-infected CF airway epithelial cells expressing the delF508 CFTR mutation exhibit higher, than in healthy cells, intracellular HRV RNA titers (Dauletbaev N et al, PLoS One 2015; Schögler A et al, Eur Respir J 2015). Susceptibility to HRV appears to be intrinsic to CFTR mutation and/or impaired CFTR function.

Research Question: We asked whether intracellular HRV RNA titers could be diminished by pharmacological correction of CFTR by the clinical drug ORKAMBI.

Methods: We grew primary CF bronchial epithelial cells under submerged conditions until approx. 80-90% confluency. Cells were then treated for 24 hours with diluent or two different combinations of lumacaftor+ivacaftor (at respective doses of 500 / 10 nM or 1000 / 100 nM) to recapitulate ORKAMBI. Thereafter, cells were inoculated for 2 hours with HRV16 at the Multiplicity of Infection of 0.1. Following inoculation, cells were washed three times to remove unattached virus, and received fresh preparations of diluent or lumacaftor+ivacaftor. At time points of 10, 22, or 34 hours post-inoculation, cell lysates were collected. Titers of intracellular HRV16 RNA (positive strand) were quantified by a hydrolysis probe-based PCR, adapted for droplet digital PCR for absolute quantification. As a positive control, Rupintrivir, an inhibitor of HRV protease C and a blocker of HRV16 RNA transcription, was used at 1 µM.

Results: Basal cultures expectedly demonstrated no detectable HRV16 RNA (< 1 copy/µl). At 10 hours post-inoculation, infected and non-treated cells exhibited a mean of 12589 copies of viral RNA/µl. Treatment with diluent or either lumacaftor+ivacaftor combinations did not diminish HRV16 RNA titers (respective means of 18197, 22909, and 14125 copies/µl). Subsequent HRV16 RNA titers (at 22 and 34 hours post-inoculation) were also not affected by diluent or lumacaftor+ivacaftor treatments. In marked contrast, Rupintrivir decreased HRV16 RNA titer early on (263 copies/µl at 10 hours post-inoculation), demonstrating the principal ability to modulate HRV infection in CF cells.

Discussion: In submerged primary CF bronchial epithelial cells, lumacaftor+ivacaftor treatment (i.e., ORKAMBI) does not diminish HRV16 RNA titer. Further studies with well-differentiated primary CF bronchial epithelial cells are underway to confirm the results from submerged cells. Specific approaches to block rhinovirus transcription may be needed to rectify the abnormal susceptibility of CF bronchial epithelium to HRV infections.

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P80

Transduction of Rhesus macaque lung following repeat dosing by AAV1

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The major hurdle with gene therapy is the development neutralizing antibodies in response to repeat delivery that could potentially block expression of enough CFTR to be therapeutic. Given that the turnover of airway epithelial cells may make gene transfer with recombinant AAV-based vectors transient, repeat dosing of AAV1 pseudotyped virus will ultimately be required. The goal is to assess whether repeat dosing of AAV1-CFTR vectors administered to primates leads to widespread gene transfer and CFTR expression. To test this, we sprayed into the airways of 2 healthy male and 2 female Rhesus monkeys between 2 and 3 years of age, 2 doses of 10^{13} vg of AAV2-1- Δ27-264-CFTR at 0 and 30 days, respectively, followed by a single dose of 10^{13} particles of AAV2-1-GFP at day 60. A similar construct Δ264-CFTR was shown to increase endogenous CFTR in Rhesus macaques via transcomplementation. Monkeys were sacrificed at day 90. This protocol was designed to evaluate the immune response to the AAV1 capsid and the transgene and the level of expression of the transduced proteins. Both males and females gained weight normally. Likewise, there were no adverse events related to the study indicating that triple dosing with AAV2-1 vectors is safe. Neutralizing antibody titers were measured in serum and reported as the highest serum dilution that inhibited AAV2-1 transduction by 50%, when compared to its own AAV2-1 vector-positive/serum-negative control. All animals used in the study had low neutralizing anti-capsid antibodies (< 5 reciprocal dilution, RD) at the beginning of the study. The levels increased in all animals to 380 ±150 (mean±SE), 30 days after the first dose. Neutralizing antibody titer increased to 1600±554, SD, 30 days after the second dose of AAV1- Δ27-264-CFTR. These antibody titers were lower than those we observed in our previous single dose experiment where four Rhesus macaques were instilled with a similar number of vector genomes of AAV1-lacZ (Firefly) and AAV5-lacZ (Renilla). In that study, anti-capsid neutralizing antibodies were 2560±640 RD for AAV1 and 17,920±2560 RD for AAV5. Samples were taken for vector genomes and mRNA expression from 17 different lung regions Tissues were fixed, and GFP expression in airway cells determined using confocal microscopy and GFP antibodies as we have done previously. In conclusion, repeat dosing of AAV2-1 into the lung of Rhesus macaques is safe but leads to increases in neutralizing antibodies against the vector capsid. Funded by CFF and NHLBI.

P81

Characterization of edited CFTR message following efficient, targeted integration of CFTR superexon to correct CF-causing variants in and downstream of exon23

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Low efficiency of gene editing and high number of CF causing mutations to be corrected are two of the major challenges of a therapeutical gene editing approach in CF. Here we combine two strategies that address each of these problems, homology-independent targeted integration (Suziki, 2016) and superexons (Bednarski, 2016). The former is shown to boost the gene editing efficiency and the latter is used to correct multiple mutations with one donor sequence.

We have previously reported the design and synthesis of a 1.78 kb CFTR superexon$^{23\text{-}27}$ comprising a splice acceptor site followed by exons 23-27 fused together and a 2A-mCherry reporter gene (Mention, 2017). The construct was incorporated into intron 22 using a Cas9 gRNA previously validated in our lab for this region (Sanz, 2017). With a single Cas9 gRNA and a single donor sequence, it should be possible to correct ~5% of all CF-causing variants including the currently non-druggable W1282X and N1303K variants.

Successful integration of the CFTR superexon$^{23\text{-}27}$ construct should result in a wild-type mRNA expressed under the control of the endogenous CFTR promoter with many of the normal regulatory features of the intact gene. Through use of the 2A linker, the fluorescent mCherry protein should also be expressed if editing is successful. Here, we report the efficient integration of the construct and expression of the reporter mCherry protein and a consequent increase in the CFTR message in human airway epithelial cell line, Calu3. FACs analysis of cells transfected with gRNA and the construct showed that 31% of transfected cells express the reporter protein, therefore are edited. When the enriched population of edited cells were cultured for 4 weeks, stable integration of CFTR superexon$^{23\text{-}27}$ was observed.

Initial semi-quantitative RT PCR experiments showed very low expression of CFTR mRNA. Based on the observation that, treatment with the demethylation reagent, AZA, increased the CFTR expression (Bednarski, 2016), we performed a four-day treatment of the cells with AZA, refreshing it daily. Results demonstrate increased expression levels of endogenous CFTR in Calu3 cells in response to AZA treatment. Moreover, we show that edited cells have a higher CFTR expression. Currently, we are using the AZA treatment in enriched population of edited cells to assess the level of edited mRNA.

Future experiments include utilization of minicircles, small circular plasmid derivatives free of any prokaryotic vector parts, to introduce the CFTR superexon$^{23\text{-}27}$ instead of the relatively big, 4,6kb plasmid. Using minicircles has two advantegous; increased editing efficiency due to small size (Suziki, 2016) and avoiding deleterious effects of insertion of large amounts of foreign bacterial DNA into human genome.

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P82

Correction of multiple Cystic Fibrosis-causing variants by CFTR superexon homology-independent targeted integration (HITI)

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Cystic Fibrosis (CF) is caused by mutations in both alleles of the CFTR gene. Whilst 90% of individuals with CF carry at least one allele with F508del there are more than 270 other disease-causing variants. The use gene editing to correct the F508del allele has been reported in cell and animal models, but the efficiency of this template-driven homology-directed repair (HDR) approach is rarely above 1% of transfected cells without drug selection (McNeer, 2015; Hollywood, 2016). The use of a superexon donor containing fused exons 11-27 as HDR template has establish the proof-of-principle for correction of multiple mutations (Bednarski, 2016) but the efficiency was even lower. Much higher gene editing efficiency can be achieved by exploiting the non-homologous end joining (NHEJ) DNA repair pathway; we have recently shown targeted excision of CF-causing deep intronic mutations of up to 40% of transfected cells (Sanz et al., 2017).

Here we describe the incorporation of two different superexons into the CFTR locus using the NHEJ-based pathway by HITI (homology-independent targeted integration). HITI results in the physical integration of a foreign DNA at a Cas9-induced double strand break in genomic DNA, and shows significantly higher levels of gene-editing efficiency compared to HDR based techniques (Suzuki et al., 2016). HITI also has the advantage over other NHEJ based integration techniques in that it prevents integration of foreign DNA in the wrong orientation, and works very efficiently in slow/non-dividing cells such as long-lasting lung epithelial cells that are potential target cells for gene editing in individuals with CF.

The superexons comprise an inverted gRNA target site, a splice acceptor site and either CF exons 11-27 or 23-27 fused as a partial cDNA linked to the 2A-mCherry reporter. We have observed targeted integration into intron 10 or 22 respectively using Cas9/gRNAs previously validated in our lab (Sanz, 2017). Analysis of the 5' and 3' integration sites shows a high level of precise integration. Successful integration of the CF Superexon 11-27 or 23-27 should result in the expression of mRNA with wild-type sequence that can potentially correct 92% or 5% of known CFTR variants respectively. We may expect that the chimeric mRNAs will be expressed under most of normal regulatory features of the intact gene, including the promoter and the intronic regions upstream to the integration site, which stays unaltered. Although integration of the Superexon sequences has been successful, initial attempts to characterize the chimeric mRNAs containing CFTR-mCherry sequences derived from the integrated superexons have been unsuccessful. To figure out if integrated sequences are correctly transcribed and spliced we have designed modified superexon sequences which contain unique primer-specific sequences to enable detection by RT-PCR (Bednarski et al., 2016) and/or short in-frame insertions to be detected by RT-PCR and FLA analysis. These variant superexons will be used to characterize the restoration of normal splicing and subsequent functional rescue of CF mutations in suitable human cell models.

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P83

Development of a nanoparticle formulation for delivery of a CRISPR/Cas9 gene therapy for Cystic Fibrosis

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Cystic Fibrosis (CF) is the most common, recessively-inherited genetic disorder, affecting around 1 in 2,500 babies born in the UK. CF affects many organ systems, however morbidity and mortality is caused mostly by progressive respiratory impairment due to mucus retention and chronic bacterial infection in the lungs. Gene therapy is an attractive therapeutic option for CF as it could target the underlying cause of the disease, rather than treating symptoms.

Here, we aim to develop a novel gene therapy for the respiratory manifestations of the disease based on gene editing with CRISPR/Cas9. This allows for precise targeting of mutations by a guide RNA targeting molecule followed by double strand DNA cleavage by the Cas9 nuclease. This approach can be used to disrupt mutation sites by indels introduced by non-homologous end joining repair of the double strand breaks, insert pieces of DNA by homology independent target integration or, in the presence of a donor DNA template, repair mutations by homology directed repair. CRISPR/Cas9 could be used to correct any kind of mutation in CFTR. However, a major hurdle to overcome in developing CRISPR-based gene editing therapies is how to deliver the technology with sufficient efficacy to the lung.

Our approach is to deliver CRISPR with a non-viral nanoparticle, previously described for *in vivo* DNA and siRNA delivery to the lung. These nanoparticles comprise peptide and lipid components, which package nucleic acids and target their delivery to epithelial cells. Gene editing of airway epithelial cells is permanent and so repeated delivery with these non-immunogenic nanoparticles could be performed until a sufficient level of genetic correction was achieved. We first optimised the nanoparticle lipid and peptide composition for transfection of primary airway epithelial cells. These primary cells are notoriously resistant to transfection but our optimal nanoparticles, formulated with GFP mRNA, achieved up to 70% transfection efficiency, as measured by flow cytometry, indicating the utility of our formulation in these cells.

We then engineered primary, human bronchial epithelial cells to stably express GFP by lentiviral transduction and used this model to compare nanoparticle formulations for knockout of GFP by CRISPR/Cas9. Nanoparticles, with different lipids and peptides, were formulated with GFP-targeting guide RNAs and Cas9 mRNA, and achieved levels of around 60% GFP knockdown by flow cytometry with optimal formulations. Nanoparticles packaging the Cas9 protein, pre-assembled with GFP synthetic guide RNA in a ribonucleoprotein complex, elicited 40% GFP knockdown. Both these transfection levels for RNA and protein based formulations were higher than that achieved by commercially available reagents.

This work provides the foundation for the development and delivery of a CF gene editing strategy *in vivo*.

P84

Cultivation and characterisation of human airway epithelial cells with potential for CFTR editing for the development of cystic fibrosis therapies

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Despite cystic fibrosis (CF) being the most common heritable disease among people with Northern European ancestry and affecting around 10,000 people in the UK alone, no cure is currently available. With drugs for its treatment showing limited efficacy and working only on patients with specific mutations, we believe that gene therapy is a promising approach to cure CF. CRISPR/Cas9 is a revolutionary gene editing system that provides a relatively easy way for precise and permanent correction of the CFTR gene.

However, since CRISPR/Cas9 delivery is still inefficient for correction of sufficient airway epithelial cells in vivo, we aim to select and expand corrected cells in vitro and subsequently deliver them to the airways as cell therapy.

We have expanded and optimised the culture conditions of different human airway epithelial cells. These include Human Fetal Tracheal Cells, ΔF508 Nasal Epithelial Cells, Normal Human Bronchial Epithelial Cells and 3849+10 C-> T Airway Epithelial Cells. Our conditions which involve culturing of the airway cells in the presence of an irradiated mouse fibroblast feeder layer, has allowed us to expand adult primary cells for over 10 passages (and more than 40 population doublings) while maintaining their ability to differentiate in vitro in Air Liquid Interface cultures (ALI). The differentiated cultures are positive for airway epithelial cell markers and differentiation markers (ciliation, mucus cell and tight junction markers). The cells also demonstrate electrical responses and the expected chloride channel activity, according to their phenotype (CF and Normal cells), in Ussing Chambers.

Additionally, we have optimised a transfection approach for these cells which yields approximately 65% GFP+ cells and developed a repair plasmid with a selection cassette which will allow correction of ΔF508 Nasal Epithelial Cells via the HDR CRISPR/Cas9 pathway without the need for single cell cloning.

Further future aims include the expansion and *in vitro* differentiation of the corrected cell population followed by the delivery of healthy, CF and corrected cells to the airways in small animal models for engraftment and repopulation.

P85

Curated database of candidate therapeutics for the activation of CFTR-mediated ion conductance (CandActCFTR)

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Background: Small molecules promoting processing, maturation and trafficking of CFTR were sought for decades and two approved compounds can be prescribed for CF patients in 2017. However, several hundred therapeutic molecules have been studied in the academic community, and the search for further therapeutic options to promote CFTR function is ongoing.

Aims: CandActCFTR is a pilot project to establish a curated database on CFTR modulating substances enabling a systems biology view based on comprehensive compound related data.

Methods: By merging data from publicly available sources, unpublished primary data and findings derived from screening efforts, we offer a community driven web-service to collect and organize available data in a user friendly interface -- for chemists, biologists and pharmacologists. The project relies on open source software and the resulting database will offer search and publishing tools freely usable upon registration. The software can be deployed locally, to collect data using the same data format and workflow tools available in the central repository instance. Later facilitating sharing either by uploading/migrating to the central repository or privacy preserving by just allowing queries to the local repository coming from the central query manager, with the options to fine tune the outgoing answer: from 'no answer at all' to summary or sharing of the full information.

Results: We created a free web system to enter and store chemical compound information and annotate with PubChem information, such as synonyms and graphical data. To enable literature knowledge (e.g. papers) to be linked to a list of chemical compounds; e.g. a PubMed search, we integrated an interface to an open source reference managing system (Zotero). Thereby, CandActCFTR allows queries, which compound (including PubChem information as synonym and structures) is referenced in which set of publication and vice versa.

Thus far we parsed 77 papers containing information about 2044 distinct chemical compounds. In direct comparison to existing data sources like ChEMBL CFTR target data sets (CHEMBL4051 and CHEMBL3992), we managed to compile a similar sized yet surprisingly also distinct set of compounds, with an overlap of only 12 compounds to the 490 compounds in the ChEMBL set.

A first analysis using our data set yielded encouraging results correlating the chemical space to systems biology space. As a hypothesis to test we inferred that outliers in chemical space should map to rather distinct biological targets; e.g. pointing to the 'autophagy-dependent' referencing publications containing rather distinct compounds, which cluster as expected.

Conclusions: So far we created an initial, robust tool set as CandActCFTR knowledge hub. We are ready to collect further data on CFTR modulating substances which than can be analyzed in context of the seed data set obtained so far. Adding more detailed information about the used assay systems/conditions and results will be included in the next step.

Additionally, integration of other resources like PubChem CFTR Target and ChEMBL target will follow, as well as applying cheminformatic methods like target structure based Constraint Network Analysis.

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P86

Restoration of CFTR function by antisense oligonucleotide splicing modulation

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A significant proportion of disease-causing mutations in humans affects pre-mRNA splicing. In Cystic fibrosis (CF), 10-15% of the mutations affect the correct splicing of the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene. Disease severity in patients carrying these mutations is highly variable, correlated with the level of aberrantly spliced transcripts. Antisense oligonucleotides (ASOs) were found to be highly efficient in modulation of the splicing pattern in several genetic diseases. We aim to develop an antisense oligonucleotides (ASOs) based therapy to modulate the level of correctly spliced CFTR. As a model we first focus on a common CFTR splicing mutation, the 3849+10kb C-to-T, which leads to inclusion of an 84 bp cryptic exon between exons 22-23 in the mature mRNA. This cryptic exon contains an in-frame stop codon that leads to degradation of a significant fraction of the mRNA by the NMD pathway as well as to the production of prematurely truncated nonfunctional proteins. We designed 2´-O-methyl phosphorothioate-modified ASOs, targeted to prevent the recognition of enhancer splice motifs in the cryptic exon or to mask the junctions between this exon and its flanking sequences. Screening of these AOSs led to the identification of several ASOs that significantly decrease the level of aberrantly spliced CFTR mRNA and increase the level of correctly spliced CFTR mRNA. Importantly we demonstrate that ASOs modulating this splicing pattern can restore the CFTR channel function in primary respiratory epithelial cells. Our results indicate that ASOs targeted to mask splicing motifs in the CFTR gene can increase the correct splicing of CFTR leading to improvement of channel function.

P87

In vitro characterization of clinical stage novel corrector PTI-801 and potentiator PTI-808 in primary airway cell models

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A phenotypic high-throughput screen that utilized a PTI amplifier to enhance the signal-to-noise ratio was performed. This strategy enabled the detection of raw hit compounds with modest functional activity that were missed in a screen of the same small molecule library that did not employ amplifier. PTI-801 is an optimized derivative of a small molecule identified using this strategy. Using immunoblotting, PTI-801 is shown to increase the levels of the mature glycosylated form of F508del-CFTR protein in primary HBE cells derived from *F508del* homozygous donors, consistent with its mechanism of action being that of a corrector. PTI-801 also increases the F508del-CFTR-mediated chloride channel activity in primary HBE cells in Ussing chamber measurements.

The corrector activity of PTI-801 shows specificity for CFTR, substantially increasing the maturation of F508del-CFTR protein, while having no impact on the maturation of a mutant form (G268V) of the related P-glycoprotein (PgP). In addition, the effect of PTI-801 on F508del-CFTR activity is maintained for 12 hours after removal of the compound, indicating that the PTI-801 functional benefit is on, or results in, a stable species of functional CFTR protein.

Like other correctors, PTI-801 is complementary with the potentiator ivacaftor, enhancing the function of F508del-CFTR in HBE cells from *F508del* homozygous donors to similar levels as those observed in cells treated with lumacaftor and ivacaftor. Unlike other correctors, under conditions in which PTI-801 is co-administered with ivacaftor, PTI-801 activity is maintained, exhibiting an enhanced *in vitro* efficacy relative to cells in which ivacaftor and lumacaftor are co-administered. In triple combinations, PTI-801 more than doubles the activity of lumacaftor and ivacaftor. This ability of PTI-801 to further enhance F508del-CFTR activity above that achieved with lumacaftor is consistent with the corrector activity of PTI-801 working through a mechanism of action that is distinct from lumacaftor.

To complement the PTI-801 corrector and PTI-428 amplifier in development at PTI, we have also developed PTI-808, a small molecule potentiator designed to rescue mutant CFTR chloride channel activity. PTI-808 enhances CFTR channel activity across partially purified channels, recombinant cell lines, and primary HBE cells from CF donors. Patch-clamp on isolated cell membranes expressing wild-type CFTR demonstrates that PTI-808 increases the open probability of the channel. Cell lines expressing recombinant G551D-CFTR showed increases in chloride current in response to acute additions of PTI-808, also consistent with PTI-808 acting through a potentiator-like mechanism on CFTR. Importantly, PTI-808 exhibits potentiator activity in Ussing chamber assays in HBE cells derived from CF donors who have gating- and conductance-defective CFTR mutations on one allele. The potency of PTI-808 between cell lines and HBE cells and between acute and chronic incubations, is consistent, exhibiting an EC_{50} of 2nM-12nM.

PTI-808 increases chloride transport in HBE cells derived from patients with one or two F508del alleles. The co-administration of PTI-808 with PTI-801and PTI-428 results in CFTR activity of 106% of normal CFTR function in F508del homozygous HBE cells, and 63% of normal CFTR in HBE cells with one allele of F508del and one allele of a nonsense mutation.

P88

Advances in an amplifier-enhanced CFTR premature termination codon readthrough high throughput screen

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Nonsense mutations are present in approximately 10% of CF patient population. In these patients, premature termination codons (PTC) lead to premature translational termination resulting in truncated or non-functional CFTR protein. A cellular quality control system, the nonsense-mediated decay (NMD) pathway, also exacerbates the defect of PTCs by promoting the degradation of mRNAs that harbor such mutations. NMD activity appears to be largely dependent on the distance relationship to introns that are downstream of the PTC, and thus is likely diminished or absent when PTCs are encoded on cDNAs in a typical reporter system used for read-through high-throughput screens.

The read-through therapeutic approach is based on the suppression of translational termination at PTCs, resulting in the restoration of full-length protein. The ability of aminoglycoside derivatives to induce the read-through of nonsense mutations suggest that it may be possible to identify a small molecule therapy that is able to induce the read-through of PTC mutations in CFTR to produce full length and functional CFTR protein.

PTI amplifiers, by stabilizing the mRNA of CFTR in a translationally-dependent, and mutation agnostic mechanism, can enhance the efficacy of read-through agents, likely owing to the increase in the amount of substrate PTC-containing mRNA. Combinations of amplifier and aminoglycosides promote the functional rescue of CFTR in G542X-derived cell lines and G542X homozygous patient-derived human bronchial epithelial cells (HBE). Based on this data, we have performed an initial high throughput screen (HTS) of 150K compounds in combination with an amplifier in a trafficking assay in FRT (Fisher Rat Thyroid) cells expressing G542X-CFTR-HRP. Screening in the presence of amplifier enhanced the effectiveness of the assay (5-fold increase in assay window), thus increasing the number of hits. Moreover, screening in the presence of amplifier uncovered modulators that would not have been identified in a standard non-amplifier HTS.

The initial screen resulted in 100 confirmed hits that were further grouped into 6 different chemotypes for testing in the Ussing chamber assay using G542X homozygous HBE cells. To enhance the very low chloride transport activity present in these cells, we employed a chloride gradient. In addition, the amplifier enhancement achieved in the screening cell line is used, as well as a corrector, lumacaftor, in order to increase the response to the compounds. All 6 chemotypes have been shown to specifically increase endogenous CFTR mRNA in G542X-HBE cells but not in F508del-HBE cells.

To improve upon this approach, PTI is developing novel PTC screening strategies that would incorporate a more physiologic context for the CFTR PTC mutations under study by including an intron downstream of the mutation. In addition, follow-up screens will be functional screens instead of trafficking screens. These modifications may also enable the identification of NMD-modulating compounds, or other mechanisms that have been missed in previous cDNA-based approaches.

P89

A novel CFTR potentiator with a distinct mutation-specific profile to existing CFTR potentiators identified using an amplifier-enhanced HTS

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There are over 2000 mutations that have been identified in the Cystic Fibrosis transmembrane Conductance Regulator (CFTR) gene. However, only 281 mutations have been characterized as CF-causing in the CFTR2 database (as of March 2017). These mutations can be divided into six different categories or theratypes. The deletion of phenylalanine at position 508 (F508del) is the most prevalent mutation among CF patients, with over 85% of patients carrying at least one allele. The F508del mutation leads to the formation of a misfolded protein that attenuates the trafficking of CFTR to the apical surface of bronchial cells of the lungs. Chemical chaperones known as correctors rescue this trafficking phenotype. However, the F508del CFTR protein also requires another form of modulation to increase the open-probability of the channel at the membrane to allow chloride transport. This is achieved using modulators known as potentiators.

Inclusion of Proteostasis Therapeutics, Inc.'s amplifier CFTR modulator in a high throughput phenotypic screen allowed the discovery of CFTR modulators that did not have sufficient activity to be identified as hits on their own, but were synergistic with the amplifier. We used the amplifier enhancement strategy to screen for potentiator molecules. In order to eliminate potentiators that also promoted the in vitro destabilization of CFTR protein during chronic exposure, we employed chronic dosing conditions in the potential phenotypic screen to discover potentiator-like molecules that lack this destabilization effect. Under this paradigm, several distinct chemical series of novel CFTR modulator were discovered.

Here we report the discovery and characterization of a novel class of CFTR modulator that has distinct characteristics from known modulators. While these modulators were selected for their activity under chronic exposure, one class of compounds also shows activity in primary HBE cells upon acute addition. The series complemented known correctors and amplifiers. In isolated cell membrane patch-clamp, they do increase the open probability of the channel, demonstrating their mechanism of action as a potentiator.

As may be expected for a potentiator, they did not synergize with known potentiators. Unexpectedly, in CFTR-mediated chloride transport assays, these compounds demonstrate a mutation-dependent efficacy that is distinct from that of known CFTR potentiators.

In summary, we report on the characterization of a new class of CFTR potentiators with characteristics distinct from known potentiators. These potentiators may represent a novel mechanism for improving mutant CFTR function.

P90

New correctors rescue F508del-CFTR activity at low nanomolar concentrations

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The deletion of phenylalanine at position 508 (F508del) of the CFTR protein is the most frequent mutation among patients with cystic fibrosis (CF). This mutation causes a severe defect in protein folding and stability, and also affects the gating behavior. The folding and stability defect can be treated with compounds known as correctors, whereas the gating defect can be overcome by compounds called potentiators (1, 2). Only one corrector has been approved for the treatment of CF patients bearing the F508del-CFTR mutation, i.e. lumacaftor (VX-809), but only in combination with the potentiator ivacaftor (VX-770). The therapeutic benefit of the combination (Orkambi®) is however limited. There is therefore the need of new, more effective correctors.

The screening of a collection of about 15,000 maximally diverse commercial compounds, using FRT and CFBE41o- cells stably expressing F508del-CFTR and the Halide-Sensitive Yellow Fluorescent Protein (HS-YFP) (3), yielded two hits, belonging to different chemical classes. Rounds of chemical modifications of the hits and functional evaluation in different assays provided information on the structure-activity relationship for each chemical class.

One of the two classes was investigated more extensively, and the medicinal chemistry work led to a number of compounds with high potency and efficacy in rescuing the activity of F508del-CFTR in bronchial epithelial cells from CF patients homozygous for the F508del mutation, as measured by short-circuit current recordings.

The best correctors showed potency in the low nanomolar range, retaining very good efficacy at the concentration of 10 nM or even lower. The data generated on the most promising correctors will be presented and discussed.

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P91

Structurally diverse Trimethylangelicin derivatives correct the primary defect in p.Phe508del-CFTR by stabilizing the first membrane-spanning domain

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The psoralen-related compound 4,6,4'-trimethylangelicin (TMA) potentiates the cAMP/PKA-dependent activation of WT-CFTR and rescues F508del-CFTR-dependent chloride secretion in immortalized as well as primary airway cell homozygous for the F508del mutation (Tamanini, 2011; Favia, 2014). We recently demonstrated that TMA, like VX-809, stabilizes the first membrane-spanning domain (MSD1) (Laselva, 2016) and enhances the interface between NBD1 and ICL4(MSD2). TMA also demonstrated anti-inflammatory properties by reducing IL-8 expression (Tamanini, 2011), so making TMA a promising agent for treatment of cystic fibrosis. Unfortunately, TMA was also shown to have potential phototoxicity and mutagenicity, when irradiated with UVA light. Due to concerns about these toxic effects, new generation TMA analogues with identical or better activity profile but reduced or absent side effects were synthesized by modifying specific structural features on the TMA scaffold, thus obtaining selected compounds with no mutagenicity and phototoxicity. Among these TMA analogues, DMA, ALLO, IPEMA and IPDMA do maintain the potentiating activity on CFTR function in FRT-YFP-G551D cells. Nanomolar concentrations of these analogues significantly rescued the F508del CFTR-dependent chloride efflux in FRT-YFP-F508del cells and CF bronchial epithelial cells. To identify the TMA binding site in CFTR, we studied the ability of TMA analogues to enhance the stable expression of varying CFTR truncation mutants in HEK-293 cells. Not surprisingly, the smallest domain that was stabilized by TMA analogues was the MSD1 in HEK-293 cells, as previously observed for TMA. In addition, we found that TMA analogues are not effective on F508del-CFTR protein that already had been stabilized by second-site mutation at the NBD1-ICL4 interface. Altogether these findings demonstrate that these TMA analogues display dual corrector and potentiator activities and bind to MSD1 domain of CFTR, to stabilize the interface between NBD1 and CL4 in order to facilitate correct folding and limit the premature degradation.

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Role of ganglioside GM1 on CFTR stabilization at plasma membrane: a new challenge for the cystic fibrosis therapy

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The new challenge for the cystic fibrosis (CF) therapy is based on the development of small molecules able to rescue the function of CFTR. Many pharmacological agents have been designed to increase the surface level of mutated CFTR (correctors), as well as its PM activity (potentiators). Unfortunately, for the most common CF-causing mutation F508del, their efficacy seems to be time-limited mainly due to a limited stability of corrected protein (1, 2). In fact, many factors contribute to PM CFTR stability, including its compartmentalization in membrane portions enriched in monosialoganglioside,1 (GM1) and multiprotein complex involving ezrin and NHERF1 (3).

Interestingly, it has been proved that in bronchial epithelial cells the lack of CFTR in the cell PM, such as in the case of the patients carrying the mutation F508del, is associated to a decreased content of GM1 (4). On the basis of these findings, we investigated the effects of the potentiator VX-770, corrector VX-809, and of the ganglioside GM1 on CFTR plasma membrane stability. We analysed CFTR expression in CF bronchial epithelial cell lines treated individually with VX-809, or VX-770 or combination of both drugs, in presence or not of GM1. In CF cells, the treatment with the corrector VX-809 induces an increase in the mature form of CFTR, effect that is reverted by the combined treatment with the potentiator VX-770, as already known (1, 2). Interestingly, when GM1 is exogenously administered to these cells, the content of the mature form of CFTR remains high even after the combined treatment with corrector and potentiator.

Consequently, we analysed the effect of VX-809 and VX-770 on CFTR scaffolding proteins, NHERF1 and ezrin, involved in CFTR stabilization. We found that the expression of both proteins has increased in cells treated with GM1 and subjected to double pharmacological treatment, with respect to the cells treated with corrector and potentiator but without GM1.

In conclusion, these results support the role of sphingolipids in the stabilization of CFTR at PM level, suggesting new therapeutic strategy for the treatment of CF by the use of GM1, molecule already proposed for the therapy of other pathologies (5).

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Exploiting a PI3Kγ Mimetic Peptide as a standalone drug to restore CFTR function, reduce inflammation and limit obstruction of the respiratory tract in cystic fibrosis

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Background and Rationale: The underlying cause of cystic fibrosis (CF) is a mutation in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR), a cyclic AMP (cAMP)-stimulated chloride channel. The consequent CFTR dysfunction primarily affects the respiratory system, where the reduced activity of the channel results in obstruction of small airways and, together with airway inflammation and infections, eventually leads to respiratory failure. Current treatment regimens for CF combine several agents delaying pulmonary dysfunction and, as a consequence, are complex and time consuming, with a tremendous impact on the quality of life of patients. Recently, a number of CFTR correctors and potentiators, improving membrane expression and gating of the channel respectively, have been developed, but their ability to rescue the basic defect of CF is still unsatisfactory.

Hypothesis and Objectives: We previously showed that phosphoinositide 3-kinase γ (PI3Kγ) acts as a scaffold protein which negatively regulates cAMP by favoring the activation of key cAMP-degrading enzymes, phosphodiesterases 3 and 4 (PDE3 and PDE4). Here, we hypothesize that targeting PI3Kγ scaffold activity enhances cAMP in airway smooth muscle, immune and epithelial cells, leading to concomitant (i) bronchodilation, (ii) anti-inflammatory effects and (iii) CFTR modulation.

Methods: We explored the ability of a cell-permeable peptide targeting the scaffold activity of PI3Kγ (Patent n° PCT/IB2015/059880 - WO/2016/103176) to function as a (i) bronchodilator, (ii) anti-inflammatory agent and (iii) CFTR modulator. A mouse model of chronic lung inflammation (OVA-sensitized mice) and human primary bronchial epithelial cells (F508del) were used.

Results: We found that, in vivo, the peptide: i) can be efficiently delivered to the lower airways by intratracheal instillation in mice; ii) significantly elevates cAMP in the lungs and iii) is devoid of the typical side effects of PDE inhibitors, such as cardiac arrhythmias and emesis. Notably, the peptide limits methacholine-induced airway hyperresponsiveness and reduces neutrophilic lung inflammation in OVA mice. In vitro, the peptide potentiates F508del-CFTR currents upon pharmacological correction with VX-809 and, unlike the gold-standard potentiator VX-770, does not interfere with channel stability in the chronic setting. In addition, the peptide is also able to stabilize and consequently increase CFTR density in the plasma membrane, Finally, in VX-809-corrected primary cells, the peptide synergizes with VX-770, by increasing its efficacy by 5 fold.

Conclusions: Overall, the results of this study demonstrate that the peptide targeting PI3Kγ may be exploited therapeutically as a new medicinal product that may offer unique advantages over current existing therapies for CF: I) three independent therapeutic benefits in a single molecule (CFTR rescue, anti-inflammatory and bronchodilator effects);

ii) high intrinsic specificity of action (due to its peptidic nature) and iii) limited side effects (thanks to the delivery by inhalation which contributes to maximize its effects on the lungs and, at the same time, reduce possible side effects typical of systemic administration).

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RNF5 inhibitors as potential drugs for cystic fibrosis basic defect

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Background: In cystic fibrosis,deletion of phenylalanine 508 (F508del) in the CFTR anion channel is associated to misfolding and premature degradation of the mutant protein. Among the known proteins associated with F508del-CFTR processing, particularly interesting is the ubiquitin ligase RNF5/RMA1. Genetic suppression of RNF5 in vivo led to attenuation of pathological phenotypes due to intestinational malabsorption in CF mice, validating the relevance of RNF5 as a target for CF.

Aim: Our aim is to identify small molecules inhibitors of RNF5 ligase activity able to mimic the effect of RNF5 silencing and dissect the molecular mechanism(s) through which RNF5 suppression leads to mutant CFTR rescue.

Methods: We generate, by homology modeling thecnique, a model of structure of the Ring domain of RNF5. This model has been used to run a virtual screening campaign to identify small molecules that can inhibit RNF5 ligase activity.

Results: In particolar, we identify a RNF5 inhibitor named inh-2. In vitro experiments demonstrate that the treatment with inh-2 modulates ubiquitylation of ATG4B, a known RNF5 target. Long therm incubation with inh-02 causes significant F508del-CFTR rescue in immortalized and primary bronchial epithelial cells deriving from CF patients homozygous for the F508 mutation.

Conclusions: Our results clearly demonstrate that RNF5 inhibition can rescue F508del-CFTR trafficking defect and that this mechanism is not only amenable in cell lines or in a murine CF model, but also in human primary bronchial epithelia, that are the main target tissue of CF treatment. These finding thus validate RNF5 as a drug target for CF and providing evidences to support its druggability.

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Anion transport mechanisms through lipid bilayers by synthetic ionophores: towards a cystic fibrosis therapy

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Cystic fibrosis (CF) is a genetic lethal disease, originated from the defective function of the CFTR protein, a chloride and bicarbonate permeable transmembrane channel. CF mutations affect CFTR through a variety of molecular mechanisms which result in different functional defects. Current therapeutic approaches are targeted to specific groups of patients that share a common functional defect. We seek to develop an innovative therapeutic approach for the treatment of CF using anionophores, small molecules that facilitate the transmembrane transport of anions. We have characterised the mechanism of anion transport of synthetic molecules based on the structure of prodigiosine, a red pigment produced by bacteria. Using ion sensitive electrodes (ISE), we measured the chloride eflux from large unilamelar liposomes upon addition of micromolar amounts of anionophores. Data is consistent with a carrier that facilitates the transport of anions through lipid membranes down the electrochemical gradient. The transport is not coupled with proton or hydroxide translocations. The selectivity sequence of the prodigiosin inspired ionophores is formate > acetate > nitrate > chloride > bicarbonate. Sulphate, phosphate, aspartate and gluconate are virtually not transported by these anionophores. The transport activity is modulated by the pH at the side where the anionophore is applied, suggesting that the ionization state of the molecule may decide the anion-anionophore interaction. These prodigiosin-derivate ionophores can also induce anion transport in living cells. Their capacity to transport chloride and bicarbonate when applied at low concentration, and low toxicity take shape as a promising starting point for the development of CF-therapy drug candidates.

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Small molecule anionophores promote transmembrane anion permeation matching CFTR activity

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Anion selective ionophores, anionophores, are small molecules capable of facilitating the transmembrane transport of anions. We have screen a large number of active anionophores and identified a family of such compounds, inspired in the structure of natural product prodigiosin, which proved highly efficient anion exchangers in model phospholipid liposomes. Their activity as anionophores in living cells was studied and chloride efflux and iodine influx from living cells mediated by these derivatives was demonstrated. These compounds were shown to permeabilize cellular membranes to halides with efficiencies close to the natural anion channel CFTR at doses that do not compromise cellular viability. Remarkably, optimal transport efficiency was measured in the presence of pH gradients mimicking those found in the airway epithelia of Cystic Fibrosis patients. These results support the viability of developing small molecule anionophores as anion channel protein surrogates with potential applications in the treatment of conditions such as Cystic Fibrosis derived from the malfunction of natural anion transport mechanisms.

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Building *in vivo* potency and duration of action and into a novel inhaled ENaC blocker, ETD001

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Inhaled drugs are typically cleared very rapidly from the airway mucosa. Inhaled amiloride, accelerated mucociliary clearance in clinical studies, but with a half-life in the airway mucosa of < 30min, it was a short acting effect which did not translate into clinical efficacy. To achieve a long duration of action in the clinic with inhaled ENaC blockers, it will be necessary to maintain compound levels in the airway lumen at a concentration that will maintain target engagement for a sufficient period of time.

The aim of the present project, was to discover novel inhaled ENaC blocker compounds. A key element of the optimisation phase was to understand lung-specific pharmacokinetics, and how these related to in vivo efficacy. To this end, two key models were used. The first employed intra-tracheal delivery of test compound into the lungs of rats with sampling of compound levels in the airway, lung tissue and plasma. Compounds were then tested for duration of action and efficacy in the sheep model of mucociliary clearance (MCC).

Over the course of this project, we discovered numerous novel and potent ENaC blockers with a range of pharmacodynamic, pharmacokinetic and physicochemical properties. Of these, ETD001 was selected as a development candidate based upon its overall profile. Notably, ETD001 was not the most potent compound, with an in vitro potency (in nM) of: 40, 31 and 30 on human, sheep and rat ENaC respectively. ETD001 did however show a long residence in the airway lumen of the rat, which was accompanied by a potent and long lasting acceleration of MCC in the sheep. Other, more potent examples of novel ENaC blockers, showed significantly greater on-target potency (< 5 nM) but failed to show efficacy in the sheep, which could be correlated with a diminished retention of compound in the airway lumen.

Based on the observations that ETD001 showed a long duration of action in vivo and that this was due to sustained compound levels in the airway lumen, we tested the hypothesis that repeat inhaled dosing would further increase the potency of the compound. After a single inhaled dose of ETD001, maximum efficacy in the sheep was observed at 13 µg/kg. In contrast, following twice daily dosing of ETD001 for 3.5 days, the maximal efficacious dose was reduced to 3 µg/kg, a 4-fold increase in potency.

In parallel and consistent with the sheep efficacy data, a 7 day repeat dose study in the rat revealed that lung levels of ETD001 were increased between days 1-7 in a dose-dependent manner. Furthermore, there was no change in blood potassium levels induced by ETD001 at any of these dose levels studied.

In summary, ETD001 is a novel inhaled ENaC blocker with a long duration of action in the airway lumen. Safety and efficacy data support a human tolerated dose that is 30-40 fold over the predicted minimum efficacious human dose. This is significant in view of the potential under-dosing of the Vertex ENaC blocker, VX-371, in a recent negative Phase 2 study.

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The ENaC regulatory peptide SPX-101 is resistant to proteolytic degradation in CF sputum

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Introduction: Mucus dehydration and decreased mucocillary clearance are hallmarks of cystic fibrosis. Airway hydration is controlled by the epithelial sodium channel (ENaC) and its natural allosteric regulator short palate lung and nasal clone 1 (SPLUNC1). It has been reported that SPLUNC1 is absent in the sputum of CF patients and that the protein is degraded by neutrophil elastase. This suggests that loss of SPLUNC1 could play a major role in mucus dehydration in CF. We have developed a novel ENaC regulatory peptide, SPX-101, that can replace SPLUNC1 function. This work investigates the abundance of SPLUNC1 in sputum from healthy and CF subjects, the stability of SPLUNC1 and SPX-101 in sputum from CF patients, and the functionality of SPX-101 after exposure to sputum.

Methods: SPLUNC1 abundance was determined by western blot analysis. Stability of SPLUNC1 in sputum was determined by western blot while stability of SPX-101 was assessed by HPLC. The function of SPX-101 after exposure to sputum samples was determined by analysis of ENaC surface density and airway surface liquid height in air liquid interface cultured human bronchial epithelial cells.

Results: SPLUNC1 protein was present in the sputum of all healthy subjects tested and absent in nine of the 10 CF subjects tested. Recombinant human SPLUNC1 was rapidly degraded in CF sputum samples. Degradation could be prevented by heat inactivation of the sputum suggesting that proteases were involved. In contrast, SPX-101 was stable in these same sputum samples. Moreover, SPX-101 which had been exposed to sputum was still able to internalize ENaC and increase ASL height.

Conclusions: SPLUNC1 protein is absent in sputum from CF patients and is likely degraded by a variety of proteases therein. SPX-101, a therapeutically-optimized peptide which mimics SPLUNC1's ENaC regulatory function, is stable in sputum samples and retains pharmacological activity thereafter. These data support the continued clinical development of SPX-101 for the treatment of CF.

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KvLQT1 K^+ channel activation improves the regeneration of human cystic fibrosis airway epithelium

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Background: Respiratory failure due to airway damage and remodelling remains the main cause of mortality in CF patients. Therefore, a better comprehension of the mechanisms involved in airway epithelial regeneration is crucial to improve their lung function. We previously observed that the regeneration of CF airway epithelium is delayed and that the regenerated CF epithelium is abnormal.

Objectives: Our aim was to determine the role of KvLQT1 channels in airway epithelial regeneration and to evaluate the impact of a combined treatment with a KvLQT1 activator and CFTR modulators on the regeneration of differentiated CF airway epithelia.

Methods: The kinetics of epithelial regeneration were evaluated by histological and immunochemistry analyses after modulation of KvLQT1 and CFTR channels using three different models: 1) an *in vitro* model of cultures from human non-CF and CF primary airway epithelial cells (AECs) at the air liquid interface (ALI), 2) an *in vivo* model of rat trachea engrafted with human non-CF and CF AECs in nude mice and 3) an *in vitro* model of ALI cultures of AECs from wild type and KO KvLQT1 mice.

Results: Chronic pharmacological inhibition of KvLQT1 channels with clofilium was associated with delayed regeneration of a differentiated and ciliated epithelium in the *in vitro* model of ALI non-CF AEC cultures as well as in the *in vivo* model of non-CF xenograft. Similarly, ALI cultures of AECs from KO KvLQT1 mice exhibited slower regeneration kinetics, compared to AEC cultures derived from wt mice. In contrast, chronic pharmacological activation of KvLQT1 channels with R-L3 improved the regeneration of a differentiated and ciliated epithelium within models of ALI cultures and xenografts with CF AECs. Importantly, a combined treatment with RL-3 and the CFTR modulators (i.e. the VX-809 corrector and the VX-770 potentiator) further increased the number of ciliated cells and also improved the apical membrane localization of CFTR in CF epithelia.

Conclusions: Our data showed that KvLQT1 activation elicits pro-regenerating effects with improved ciliated cell differentiation and apical CFTR localization. Thus, KvLQT1 channels could be identified as interesting therapeutic targets to favour CF airway epithelial regeneration.

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Development of novel ABCC4 inhibitors for potentiation of CFTR in human airway epithelial cells

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Background: CFTR activity is potentiated by phosphorylation by cAMP-dependent PKA. Intracellular cAMP levels are regulated by adenylyl cyclases, phosphodiesterases, and extracellular transport. We have recently demonstrated the function of ABCC4 as an extracellular cAMP transporter in human airway epithelial cells (Gold-2016-Mucosal Immunology). We next demonstrated that pharmacological inhibition of ABCC4 can potentiate CFTR activity in human airway epithelial cells (Ahmadi-2017-Genomic Medicine), validating the pursuit of novel ABCC4 inhibitors for Cystic Fibrosis therapy.

Aims: Perform an in silico screen with in vitro validation to identify candidate ABCC4 inhibitor molecules.

Methods: We performed in silico screening of the eMolecules database using ligand similarity models. Candidates were purchased from commercial vendors, screened at 10uM in an in vitro cAMP efflux assay with human airway epithelial cells, with top candidates undergoing a dose-response analysis (0.1,1,10,100uM). In vitro analysis of CFTR function was assessed in top candidates.

Results: Over 3 million compounds in the eMolecules database were screened resulting in identification of 39 compounds of interest. Seven compounds reduced forskolin-stimulated cAMP efflux signal by greater than 50%, 26 reduced cAMP efflux signal to between 50% and 100%, and 6 compounds increased cAMP efflux signal. The IC50 values for the top candidates ranged from 0.8 to 6uM. Using IC50 values, CFTR was potentiated between 0% to 8.7%.

Conclusions: Using *in silico* and *in vitro* screening assays, we identified novel ABCC4 inhibitors that potentiate CFTR in human airway epithelial cells. Our findings complement our recent demonstration that ABCC4 inhibition in G551D variants increases CFTR activity beyond ivacaftor alone (Ahmadi-2017-Genomic Medicine). Our results form the basis for a drug development program aimed at developing novel ABCC4 inhibitors as add-on therapies for treatment of Cystic Fibrosis.

P101

Potassium channels as therapeutic targets in the repair of human cystic fibrosis airway epithelium in the presence of *Pseudomonas aeruginosa* exoproducts

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Background: In cystic fibrosis (CF), an efficient repair and regeneration of the damaged airway epithelium is necessary for the restoration of lung integrity and function. Our work previously highlighted a repair deficiency of CF epithelia, due to both the basic CFTR default as well as a deleterious effect of bacterial infection. Our data also unveiled that CFTR rescue with correctors significantly enhances airway epithelial repair, but this CFTR rescue and repair improvement are dampened by bacteria. Furthermore, previous studies from our laboratory revealed that another class of ion channels, i.e. K^+ channels, also regulate the repair of respiratory epithelia.

Objectives: Our work aimed to:

1) determine the expression and localization of K^+ channels (KvLQT1 and K_{ATP}) in non-CF and CF airway epithelial cells (AECs) and

2) assess a potential beneficial effect of KvLQT1 and K_{ATP} activators, in combination with CFTR modulators on airway epithelial repair, in the presence of *P. aeruginosa* (*P.a.*) exoproducts.

Methods: The wound repair rates of CF and non-CF AEC epithelia in a 2D model (human primary AEC monolayers) and a 3D model (differentiated primary AEC cultures at the air liquid interface (ALI)) were evaluated by time-lapse videomicroscopy. The AEC cultures were treated with a KvLQT1 activator (R-L3), alone or in combination with a K_{ATP} activator (pinacidil) and/or CFTR modulators (i.e. the VX-809 corrector and the VX-770 potentiator). Their effects on the repair rates were tested in the absence or presence of *P.a.* exoproducts. The transepithelial resistances of AEC differentiated cultures were measured before wounding and after different time points of repair (24h, 36h, 72h and 1 week after injury).

Results: We first determined that KvLQT1 and K_{ATP} K^+ channels were expressed in non-CF and CF AECs and are localized at both the apical and basolateral membranes. The presence of *P.a.* exproducts did not affected their expression levels.

In non-CF AEC cultures, KvLQT1 activation, with R-L3, significantly enhanced the repair rates; this effect was prevented by the KvLQT1 inhibitor, clofilium.

Similarly, exposure to the KvLQT1 activator improved the repair of CF AEC epithelia. The K_{ATP} activator (pinacidil) elicited an additive effect. Moreover, the addition of the CFTR corrector and potentiator further increased CF AEC epithelial repair. Interestingly, this combined treatment (R-L3+pinacidil+VX-809+VX-770) significantly improved the repair rates, despite the presence of infection.

Conclusions: Our data highlighted a beneficial role of K^+ channel activators on the repair of non-CF and CF airway epithelia, despite the presence of infection. Combined treatments with CFTR and K^+ channel modulators could thus be an interesting strategy to improve the repair processes of airway epithelia in CF.

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Red ginseng increases trans-epithelial chloride transport in CF and reduces *Pseudomonas aeruginosa* biofilm formation

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Background: Abnormal chloride (Cl^-) transport has a detrimental impact on mucociliary clearance (MCC) in both cystic fibrosis (CF) and non-CF chronic rhinosinusitis (CRS). In CF patients, TMEM16A has been proposed as a potential drug target to compensate for the abrogated CFTR function. Therefore, identifying potent and specific modulators of the TMEM16A channel can be crucial. A recently published study (Guo et al., 2017) demonstrated that Ginsenoside Rb1 (GRb1) can activate TMEM16A directly in a dose-dependent manner. Ginseng (GRb1, major component) is a medicinal plant noted to have a number of anti-inflammatory properties. The objectives of the present study are to assess the capability of red ginseng aqueous extract (RGAE) to promote transepithelial Cl^- secretion in nasal epithelium and to inhibit *P. aeruginosa* biofilm formation.

Methods: Wild type (WT) and transgenic $CFTR^{-/-}$ primary murine nasal septal epithelial (MNSE) cultures were pharmacologically manipulated in Ussing chambers to measure the impact of RGAE on vectorial Cl^- secretion. Effects on ciliary beat frequency (CBF) were also measured. Biofilm formation of the PAO1 strain of *P. aeruginosa* incubated with and without RGAE was quantified by crystal violet staining.

Results: RGAE (at 200 µg/ml Ginsenosides) significantly increased Cl^- transport [measured as change in short-circuit current (ΔI_{SC} = µA/cm^2)] when compared to vehicle control in WT MNSE (35.1±3.1 vs 0.1±0.2, $p < 0.05$) and $CFTR^{-/-}$ MNSE (32.6±2.0 vs 0.2±0.3, $p < 0.05$) indicating effects are independent of CFTR. CBF (fold-change/baseline) was significantly increased compared to control in WT MNSE (2.05±0.2 vs 1.25±0.0, respectively $p < 0.05$). In patch clamp analysis, RGAE significantly stimulated the UTP response more than 10 times. RGAE markedly reduced PAO1 biofilm formation compared to controls (optical density at 590nm = 0.18±0.03 vs 0.28±0.02, $p < 0.001$).

Conclusion: RGAE significantly activates transepithelial Cl^- secretion, equally in both WT and CFTR-knockout MNSE cultures, while inhibiting PAO1 biofilm formation. These findings suggest RGAE has therapeutic potential for both CF and non-CF CRS with *P. aeruginosa*. Further *in vivo* studies are planned.

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Cationic antimicrobial peptides: an alternative and/or adjuvant to antibiotics active against multidrug-resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa*

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Background: Methicillin-resistant *Staphylococcus aureus* and multidrug-resistant *Pseudomonas aeruginosa* are becoming difficult to treat with conventional antibiotics while cationic antimicrobial peptides (CAMPs) represent promising alternative options.

Aim: Examine the bactericidal effects of four CAMPs on clinical strains of *S. aureus* and *P. aeruginosa* and whether antibiotic resistance interferes with these effects. We also aimed to investigate whether the strains used develop resistance mechanisms to CAMPs.

Materials/methods: The effects of four CAMPs (LL-37: human cathelicidin, CAMA: cecropin(1-7)-melittin A(2-9) amide, magainin-II and nisin) were investigated on clinical and laboratory *S. aureus* (n=10) and *P. aeruginosa* (n=11) showing either susceptible or resistant profiles to antibiotics. Minimal inhibitory concentrations (MICs), minimal bactericidal concentrations (MBCs) and bacterial survival rates (2 hours post treatment) were determined using the microbroth dilution technique. The antipseudomonal effects of the antibiotics colistin or imipenem combined to LL-37 or CAMA were also studied. Plus, resistance to gentamicin, LL-37 or CAMA was examined on two *P. aeruginosa* strains over one month.

Results: The results revealed MICs between 4 µg/ml and >128 µg/ml for LL-37 and CAMA, respectively. MBCs were either equal or two-fold greater than MICs with no significant differences between antibiotic susceptible or resistant strains. MICs of imipenem decreased up to 4-fold and MICs of colistin decreased up to 8-fold when antibiotics were combined with LL-37 or CAMA. Induced resistance to LL-37 and CAMA was transient and much lower than the induced resistance to gentamicin.

Conclusions: This study revealed the potent and rapid antibacterial effects of tested CAMPs on both laboratory and clinical strains of *S. aureus* and *P. aeruginosa* either susceptible or resistant to antibiotics. Most importantly, CAMPs improved the efficacy of antibiotics and were associated with transient and low levels of induced resistance on tested strains. Thus, these CAMPs can be proposed as potential therapeutic tools to treat bacterial infection in patients with cystic fibrosis.

P104

Targeting IL-17-producing T cells attenuates the severity of *Pseudomonas aeruginosa* lung infection

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Excessive airway neutrophilia and submucosal lymphocytosis are key hallmarks of the host response to *Pseudomonas aeruginosa* in cystic fibrosis (CF) patients. Recent studies suggest that IL-17-producing T cells may play a key role in the response to *P. aeruginosa* infection and their suppression may provide clinical benefit in CF. Selectively targeting IL-17-producing T cells during the development of chronic *P. aeruginosa* infections allows for evaluation of the therapeutic strategy to interfere with immune mechanisms decreasing harmful inflammation, with the potential to limit exacerbations of bacterial infection. We recently developed a unique mouse model of chronic *P. aeruginosa* infection that reproduces several hallmarks of CF lung disease, including chronic airway inflammation and tissue damage (Cigana et al, Sci. Rep. 2016). This model was instrumental to demonstrate that IL-17A levels and IL-17-producing T cells were enriched in the lungs of C57Bl/6 mice, while IFN-γ- and IL-4-producing T cells were absent, indicating the involvement of type 17 rather than type 1 and 2 immunities during *P. aeruginosa* chronic infection (Lorè et al, Sci. Rep. 2016). Taking into consideration these results, we explored the therapeutic potential of CPI203, a selective small molecule inhibitor of the BET (bromodomain and extra-terminal domain) family of proteins, and GSK805, an inverse agonist of RORgt, the key transcription factor for IL17-producing T cells. CPI203 and GSK805 molecules were evaluated at early (two days) and advanced (two weeks) stage *P. aeruginosa* infection during the development of chronic colonization. Daily treatment with CPI203 in mice decreased incidence of chronic colonization, indicating a role of CPI203 in promoting host resistance to *P. aeruginosa*. In addition, CPI203 treatment reduced levels of cytokines/chemokines and infiltrating leukocytes, including neutrophils, thus ameliorating host immunopathology, without increasing the bacterial burden. In contrast, GSK805 treatment did not affect the level of infection or the host immune response at early or advanced stage *P. aeruginosa* infection. A detailed and extensive characterization by FACS analysis of the IL-17-producing cellular subsets modulated by CPI203 treatment is ongoing. Overall, our results support the further evaluation of BET bromodomain inhibition as a point of potential therapeutic intervention to reduce harmful inflammatory response without compromising the host defense during *P. aeruginosa* pulmonary infections in CF.

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P105

Treating acute and chronic *Pseudomonas aeruginosa* infection: what can we learn from mouse models?

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Objectives: Most animal models used to predict efficacy of marketed antibacterials involve the acute infection with complex models of chronic pneumonia rarely used. This generates serious concerns for the predictive power of previous animal models in the treatment of patients with long-term infection. We tested the efficacy of marketed antibacterials in acute vs chronic murine models of infection, using different administration routes (aerosol vs intranasal vs subcutaneous) and different treatment schedules (soon after infection vs during chronic colonization). We intend generating recommendations and standards for industry and academia for *P. aeruginosa* drug development.

Methods: MICs of tobramycin (TOB) and colistin (COL) were measured for reference (PAO1: TOB:0.25ug/ml; COL:0.125ug/ml) and clinical *P. aeruginosa* strains (RP73: TOB:2ug/ml; COL:0.125ug/ml). C57BL/6 mice were infected with planktonic *P. aeruginosa* PAO1 or RP73 embedded in agar beads by intratracheal instillation (i.t.). Treatment started soon or days post-infection by Penn-Century aerosolizer, intranasal (i.n.) or subcutaneous (s.c).

Results: In acute infection, single-dose of TOB was significantly effective at 6 h post-treatment reducing PAO1 bacterial load of 4 log10 CFU after aerosol administration (TOB: 2mg/kg) or s.c. (20mg/kg) and only 1 log10 CFU reduction by i.n. (2mg/kg) compared to placebo-treated mice. Treatment with aerosol (1mg/kg) or s.c. (10mg/kg) COL reduced significantly 2 log10 CFU while i.n. delivery was ineffective vs placebo. TOB and COL aerosol or s.c. treatment significantly reduced total cells, particularly neutrophils, in the bronchoalveolar lavage fluid (BALF) compared with i.n. or placebo-treated mice.

Chronic infection was induced in mice with the RP73 strain embedded in agar-beads, as we know that this well-established, characterized murine model mirrors several hallmarks of CF lung disease (Cigana, Sci Rep. 2016). In chronic infection, mice treated daily for 7 days by aerosol TOB (TOB: 16mg/kg) or s.c. (TOB: 160mg/kg) starting soon after infection showed significantly higher and faster body weight recovery when compared with placebo-treated group. At 7 days, TOB treatment by aerosol or s.c. significantly reduced lung CFU of 4log10 and total cells, particularly neutrophils and macrophages. When a similar schedule of 7 treatments was started days after infection in the presence of established chronic damage, TOB failed to eradicate *P. aeruginosa* and reduce inflammation, with mice not recovering body weight compared to placebo-treated group. Furthermore, prolonged treatment by aerosol TOB may exacerbate inflammation.

Conclusion: Our mouse infection model mimics the different phases of acute and chronic *P. aeruginosa* lung infection in CF patients, with clear measurable readouts under antibiotic treatments. In particular, our findings point towards the need for multiple animal models and different treatment schedules to optimally translate the results to the clinic.

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P106

NX-AS-401: a quorum sensing inhibitor that reduces virulence factor production, disrupts established biofilms and enhances the effect of tobramycin in multiple clinical isolates

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Background: It is well established that bacterial biofilms limit the effectiveness of conventional antibiotics in patients with CF, leading to chronic infections that are difficult to eradicate. Quorum sensing (QS) systems play a key role in the formation of these biofilms and adaptations by bacteria in the lung environment lead to mutations of QS regulator genes. Further tissue damage is caused by biofilm associated production of virulence factors, including LasB, pyocyanin, rhamnolipid, alginate and CFTR inhibitory factor (Cif)(e.g. Shaan et al., 2016). These virulence factors have been found to inhibit the natural host immune response and modulate CFTR activity. Exoproducts from *Pseudomonas aeruginosa* have been found to reduce the impact of CFTR modulators, with effects dependent on the *las* QS system (Maille et al., 2017).

Inhibiting QS and production of virulence factors in *P.aeruginosa* provides a novel treatment approach for chronic infections and can reduce the impact of infection on the lung and CFTR function. Neem Biotech's lead compound, NX-AS-401, is a QS inhibitor (QSI), that reduces the production of virulence factors across multiple clinical isolates, and enhances the efficacy of antibiotics currently used to treat chronic CF infections.

Objectives: Demonstrate QSI activity and inhibition of virulence factors by NX-AS-401 in multiple isolates of *P.aeruginosa*. Determine effects of NX-AS-401 and/or Tobramycin on established biofilms.

Methods: A *P.aeruginosa* biofilm model in Artificial Sputum Media (ASM) was used to investigate the effect of NX-AS-401 across strains. Timed expression of QS genes (*lasR, rhlR* and *pqsR)* and virulence factor genes (*lasB, rhlA, algD, phzF* and *cif*) was quantified using RT-qPCR. The production of virulence factors, pyocyanin, alginate and rhamnolipid, were assessed spectrophotometrically using the carbazole method, methylene blue and the orcinol assay respectively.

Glass coupons were inoculated with *P.aeruginosa* (OD_{600} of 0.1) and grown statically for 6hrs then incubated with gentle agitation for 24 hrs. The effect of NX-AS-401 treatment on biofilms alone and in combination with tobramycin was monitored by counting colony forming units (CFU) and structurally assessed by confocal laser microscopy.

Results: NX-AS-401 treatment of mature biofilms in ASM resulted in downregulation of QS genes *lasR, rhlR* and *pqsR* and virulence factors *lasB, rhlA, phzF and algD*, with effects still evident 48 hrs following treatment ($P< 0.05$).

Quantification of exoproducts showed a 44-72% reduction in pyocyanin across isolates 48 hrs following treatment, with alginate and rhamnolipid showing similar patterns at 24 hrs.

NX-AS-401 disrupted established biofilms and enhanced the effect of Tobramycin on CFU, with confocal imaging showing greater accessibility of antibiotic through-out the biofilm.

Conclusions NX-AS-401 has QSI activity against multiple clinical isolates of *P.aeruginosa,* inhibiting the expression of genes known to have a role in biofilm formation and virulence.

Treatment with NX-AS-401 reduces exoproducts associated with lung tissue damage and the regulation of CFTR.

NX-AS-401 disrupted established biofilms and enhanced the antibiotic activity of Tobramycin against clinically relevant strains of *P.aeruginosa*.

Neem Biotech has been granted Orphan Drug Designation by the FDA for treatment of *P.aeruginosa* lung infections in CF patients, using NX-AS-401 as an adjunct to conventional antibiotics.

P107

Development of nanoparticles for silencing the beta-glucocerebrosidase GBA2 as a promising tool to reduce cystic fibrosis lung inflammation

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Cystic fibrosis (CF) lung disease is characterized by progressive chronic infection and inflammation of the airways, which represents the major cause of mortality in patients.

Current anti-inflammatory strategies for the treatment of CF pulmonary disease are limited, thus the identification of additional molecular targets for therapeutic intervention represents one of the main challenges. In previous work, we found that the non-lysosomal beta-glucocerebrosidase GBA2 plays a key role in the regulation of the pro-inflammatory state of CF cells, as well as, the inflammatory response after *Pseudomonas aeruginosa* infection. Indeed, lowering the expression of GBA2 in CF cells exposed to PAO1 strain by siRNA results in a reduction of IL-8 expression in both uninfected and infected cells (1).

With the aim to exploiting the anti-inflammatory effect of the GBA2 silencing, we developed new nanoparticles (NP) for the delivery of specific siRNA. In particular, we considered lipid-based NP carrying siRNA pre-complexed or not with protamine. First, we performed the biophysical characterization of these nanoparticles. Then, we proceeded with the evaluation of their stability and transfection potential performing time course experiments in CF bronchial epithelial cells. The results obtained showed an efficacy on GBA2 silencing up to 8 days without any cell toxicity.

We also characterized the lipid and enzymatic profile of primary bronchial epithelial cells differentiated at the air liquid interface. Interestingly we found that GBA2 is mainly expressed at the apical membrane supporting GBA2 as a promising target for a new anti-inflammatory therapy based on the use of siRNA-delivering NP. Undergoing studies are aimed to evaluate the ability of NP to penetrate the mucus produced by these cells.

References: 1. Loberto, N., et al. (2014) GBA2-encoded beta-glucosidase activity is involved in the inflammatory response to Pseudomonas aeruginosa. PLoS One 9, e104763

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P108

Enhanced mucus diffusion with cationic nanoparticles by an alginate oligomer

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Nanoparticles enable delivery of therapeutics to the airways of the CF lung including nucleic acid therapeutics and small molecule drugs. The airway mucus layer is an effective barrier for excluding bacteria and viruses from the lung, however, it is equally effective as a barrier to nanoparticle therapeutics. We have previously described cationic nanoparticle --mediated delivery of siRNA to the lungs in mice, and of plasmid DNA (pDNA) to airways of mice, rats and pigs. Since mucus presents a physical barrier to delivery we propose that we might further enhance efficiency of transfection in the lung by improving the ability of the nanoparticles to cross the mucus barrier. Mucus comprises a mesh like structure of mucin glycoproteins, which carry a strong net-negative charge. We hypothesised, therefore, that nanoparticles with a net negative charge might diffuse more readily through the mucus barrier and furthermore that addition of mucus modulating agents might also enhance mucus mobility of nanoparticles, particularly in the more viscous CF mucus. To test these ideas, we prepared fluorescently-labelled siRNA nanoparticles with net negative surface charges (anionic) or net positive surface charges (cationic). Both formulations were mixtures of cationic targeting peptides combined with various lipids to modulate the surface charge. Size and charge were determined on a NanoZS Zetasizer to confirm size and charge of the nanoparticles then applied to the surface of a static mucus barrier model comprising a layer of CF or non-CF mucus on a semi-permeable transwell membrane with a buffer collection chamber underneath. Buffer samples were collected at intervals and fluorescence measured. The rate of accumulation of fluorescence in the lower chamber was used to calculate diffusion rates. Experiments confirmed that anionic formulations diffused more rapidly in mucus than the cationics. Unlike other studies, PEGylation did not enhance diffusion of either cationic or anionic formulations. Addition of a low molecular weight guluronic-rich alginate oligomer (OligoG) with mucus modulating properties, had beneficial effects for cationic formulations, however, an alginate oligomer comprising mannuronic residues (OligoM) enhanced more significantly the diffusion of the cationic formulations. Neither of the alginate oligomers affected diffusion of the anionics. Further analysis of size and charge showed that the OligoM interacted with the cationic nanoparticles, reversing their charge. Moreover, their size was significantly reduced by both OligoM, and Oligo G. Electron microscopy analysis of the collected cationic nanoparticles with or without OligoM, revealed that after transit through the mucus they remained intact, spherical nanoparticles.

In conclusion, mucus diffusion, surprisingly, was not improved by PEGylation of the lipid/peptide nanoparticles used in this study. Anionic nanoparticles diffuse better, due presumably to reduced interactions and entrapment in the mucin mesh. Cationic, but not anionic diffusion was enhanced by pre-treatment of the mucus with OligoM, but not OligoG. This may be due to differences in the interaction of the two alginate oligomers with the nanoparticle. This study indicates possible methods to enhance nanoparticle transfection efficiency in the lung by enabling the nanoparticles to better overcome the mucus barrier.

P109

Stilbenoids from vine as treatment for Cystic Fibrosis ?

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Background: Cystic fibrosis (CF) is an inherited disease caused by mutations in the gene encoding the CFTR chloride channel. CFTR dysfunction leads to accumulation and dehydration of the respiratory mucus in the lung, associated with chronic inflammation and infections. Airway epithelium remodeling is frequently observed, characterized by squamous metaplasia, basal and goblet cell hyperplasia, and a decreased number of ciliated cells. The vine stilbenoids, through their multiple properties, are ideal candidates for the development of a new therapeutics to improve epithelial regeneration.

Aim: The aim of our project is to determine if resveratrol and its derivatives (pallidol, δ and ε viniferins), alone or in combination, are able to improve the CF airway epithelial wound healing and to correct the CF epithelial remodeling and defective functionality.

Methods: Airway epithelial regeneration was studied in air-liquid interface (ALI) cultures of primary non-CF and CF human nasal epithelial cells. Stilbenoids were produced from vine cells, then purified and chemically characterized. Cytotoxicity assays were performed in ALI cultures chronically treated or not (from ALI D0 to D35) with the different stilbenoids. Their effects on ciliated cell differentiation were analyzed at ALI D15 and D35 after quantification of Arl13+ cells. At ALI D35, basal and goblet cell number were determined by counting CK13+ and MUC-5AC+ cells. Wound-healing assays were done on primary epithelial cell monolayers pretreated or not for 12h with stilbenoids, and cell proliferation was studied by quantifying Ki67+ cells.

Results: Cytotoxicity assays showed that resveratrol, pallidol, δ- and ε-viniferin can be used at 30, 20, 5 and 5µM, respectively. The wound closure of non-CF and CF epithelia was slower after treatment with resveratrol, pallidol and δ-viniferin, whereas ε-viniferin was not effective. ALI non-CF cultures treated with 30µM of resveratrol seemed to present more ciliated cells than control cultures at ALI D25 and D35. Analyses of stilbenoids-treated regenerated epithelia are currently in progress.

Conclusion: In conclusion, stilbenoids seem to alter differently the various stages of the airway epithelial regeneration.

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P110

Modulation of cellular membrane properties as a potential therapeutic strategy to heal pulmonary obstructive diseases

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Maintaining the equilibrium between saturated and unsaturated fatty acids within membrane phospholipids (PL) is crucial to sustain the optimal membrane biophysical properties, compatible with selective organelle-based processes. Lipointoxication is a pathological condition under which saturated PLs tend to accumulate within the cell at the expense of unsaturated species, with major impacts on organelle function. Here, we show that human bronchial epithelial cells extracted from lungs of patients with Obstructive Pulmonary Diseases (OPDs), *i. e.* Cystic Fibrosis (CF) individuals and Smokers, display a characteristic lipointoxication signature, with excessive amounts of saturated PLs. Reconstitution of this signature *in cellulo* and *in silico* revealed that such an imbalance results in altered membrane properties and in a dramatic disorganization of the intracellular network of bronchial epithelial cells, in a process which can account for several OPD traits. Such characters include Endoplasmic Reticulum-stress, constitutive IL8 secretion, bronchoconstriction and, ultimately, epithelial cell death by apoptosis. We also demonstrate that a recently-identified lipid-like molecule, which has been shown to behave as a "membrane-reshaper", counters all the lipointoxication hallmarks tested. Altogether, these insights highlight the modulation of membrane properties as a potential new strategy to heal and prevent highly detrimental symptoms associated with OPDs.

P111

New research opportunities for CF scientists: EU-OPENSCREEN and CORBEL

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The academic Chemical Biology initiative EU-OPENSCREEN (www.eu-openscreen.eu) integrates high-capacity screening platforms and medicinal chemistry groups throughout Europe. Researchers from academic institutions, SMEs and industrial organisations can access the rationally selected EU-OPENSCREEN compound collection, comprising up to 140.000 commercial and proprietary compounds collected from European chemists, to develop novel molecular tool compounds for the validation of novel biological targets, in collaboration with its partner screening platforms and medicinal chemistry groups.

EU-OPENSCREEN and other biomedical research infrastructures joined forces in the CORBEL project (www.corbel.project.eu) to offer scientists access to a wide range of technology platforms, resources and services in the fields of medical and biological imaging (www.eurobioimaging.eu), structral biology (www.structuralbiology.eu), mouse models (www.infrafrontier.eu), clinical resarch (www.ecrin.org), databases (www.elixir-europe.org), biobanks (www.bbmri-eric.eu), microbiology (www.mirri.org), marine biology (www.embrc.eu), systems biology (http://project.isbe.eu), translational research (https://eatris.eu).

P112

The CF Canada-sick kids program in individual CF therapy: A resource for the advancement of personalized medicine in CF

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BACKGROUND: Variation in CF disease severity is conferred not only by different CFTR genotypes, but also by modifier genes and environmental factors. Therapies targeting certain CFTR mutants have been approved, yet variations in clinical response highlight the need for an individualized approach, utilizing robust patient specific genetic and tissue-based testing platforms that are predictive of therapeutic outcomes to the approved and next generation compounds advancing through the drug discovery pipeline.

METHODS: CF Canada-Sick Kids Program in Individual CF Therapy (CFIT) is generating a "first of its kind", comprehensive resource possessing subject-specific cell cultures and data that will enable modeling of therapeutic responses. This resource consists of: 1) nasal cells from drug naïve patients suitable for culture and the study of drug responses in vitro, 2) matched gene expression data obtained by sequencing the RNA from the primary nasal tissue 3) whole genome sequencing of blood derived DNA from each of the 100 participants, 4) induced pluripotent stem cells (iPSCs) generated from each participant's blood sample, 5) CRISPR-edited isogenic control iPSC lines for a subset of the participants and 6) prospective clinical data from patients treated with CF modulators clinically. The development of this resource involves a multidisciplinary team with expertise in: creating and curating patient-specific biobanks, statistical genetics, genetic epidemiology, state-of-the-art technologies for personalized CF disease modeling and therapy discoveries using a combination of primary and stem cell-based assays, therapeutic drug trials and clinical care.

RESULTS: Since October 2015, we have recruited 57 of the 100 patient donors to the CFIT: 100 Cell Line Project. Consequently, we have biobanked primary airway (nasal) cultures and iPS cell lines from individuals homozygous for F508del, heterozygous for F508del and a minimal function mutation, and several rare homozygous mutations. Mid-to high-throughput methods have been established to conduct in-vitro clinical trials enabling the profiling of drug responses for multiple individuals simultaneously. Whole genome and RNA sequencing along with the corresponding informatics will soon be complete for each of these individuals.

CONCLUSIONS: We anticipate that this resource will enable testing and comparison of novel therapeutic interventions targeting F508del-CFTR, therapy discovery for rare CF causing mutations and modeling of pathogenesis and therapy in tissues that were previously inaccessible for study but can be generated from induced pluripotent stem cells.

Funding for the CFIT Program is provided through CF Canada and the SickKids Foundation.

P113
Exploring CF through variation landscapes

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Defining the impact of variation on inherited disease contributing to human health remains a major challenge in the era of human genome sequencing efforts. To understand the transformation of the genotype to the phenotype, we apply a machine learning (ML) approach to capture variation in the human genome of the CF population in the context of function. ML generates landscapes that predict the biologic organization of the entire CF protein fold and its impact on onset and progression of CF at atomic resolution. Emergent landscape design features reveal an unanticipated level of variant-sensitive fold dynamics that specify unique function in a cell, tissue and individual specific manner. Population-based variation landscapes inform us how to tailor management of CF as a precision medicine initiative in the clinical setting.

P114

Dynamic changes of DNA methylation and pulmonary disease in Cystic Fibrosis

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Lung disease is variable among cystic fibrosis (CF) patients and equally affected by genetic and environmental factors. In airway chronic diseases, external pollutants and the endogenous stress generated by the inflammatory and immune responses alter the epigenome of the affected tissues. No study has addressed genome-wide DNA methylation patterns in CF. Using the 450K BeadChip, we profiled DNA methylation in nasal epithelial cells collected from CF patients homozygous for the *CFTR* p.Phe508del mutation and healthy controls. We replicated DNA methylation changes at specific CpG sites by pyrosequencing. CF patients with forced expiratory volume in 1 second percentage predicted values corresponding to the top and bottom quartiles were classified as mild and severe, respectively. In NEC samples from 32 CF patients and 16 controls, we detected substantial DNA methylation differences up to 55% (median b change 0.13; IQR: 0.15-0.11) using the 450K BeadChip and up to 26% in selected regions analysed by pyrosequencing. DNA methylation differences at six CpG sites were validated in the same discovery set of subjects and replicated in a small independent confirmatory set. DNA methylation levels differed between mild and severe CF patients and correlated with lung function at fifty CpG sites. Differentially methylated CpG sites were overrepresented in enhancers and enriched in predicted binding sites for factors that regulate the transcription of genes involved in the immune function and in the integrity of the epithelium. Among them, EHF and ELF5, are encoded by known CF lung modifier genes. Our study (i) points to the importance of genes responsible for the integrity of the epithelium and the inflammatory and immune responses to explain lung disease variability in CF, (ii) highlights new candidate genes potentially involved in lung disease severity, while it corroborates the role of known modifier genes, and (iii) suggests that CF-associated dynamic changes of DNA methylation are prominent in transcriptionally active genomic regions.

P115

Personalized medicine for cystic fibrosis: functional characterization of rare CFTR mutations and their response to modulators in patient-derived materials and cellular models

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Background: Although most of the ~2,000 CFTR gene mutations so far described are presumed to cause CF, the respective functional defect is still unclear for most of them, thus posing considerable challenges to the establishment of a CF diagnosis/prognosis. Elucidation of the molecular and cellular defects associated with these mutations in patient-derived materials combined with data from physiologically-relevant cellular models will of high relevance not just for prediction of disease outcomes, but also to determine effectiveness of CFTR modulator therapies. CFTR mutations are thus grouped into 7 theratypes.

Aim: Here we aim to assess the molecular/functional CFTR defect associated with 12rare mutations both in patient-derived materials and cellular models, and also to determine their responsiveness to CFTR modulators in a personalized fashion.

Methods: CFTR basal activity or after CFTR modulators (potentiator VX-770 alone or with correctors VX-809 or VX-661) was determined by Ussing chamber measurements in rectal biopsies and nasal cells from CF patients with different mutations (P205S, R334W, R347P, H1079P, D614G, N1303K, G85E, 2789+5 G>A, 3120+1 G>A, 3272-26 A>G, 3849+10Kb C>T and D1152H), as well as in novel CFBE-based cell lines stably expressing these mutants. Intestinal organoids were analyzed by the forskolin-induced swelling (FIS) assay. Additional approaches (Western blot and immunofluorescence) were also performed in cellular models to assess processing/plasma membrane (PM) localization.

Results and Discussion: Data from both rectal biopsies and organoids showed residual function for P205S, R334W, D614G, 2789+5 G>A, 3849+10kbC>T and D1152H and but no function for G85E, H1079P, N1303K nor 3120+1 G>A (all with a class I/II in the other allele). Data in the cellular models show that P205S and H1079P cause a processing defect while R347P and R334W have normal processing. Function of P205S-CFTR was rescued by VX-809 or VX-661 with VX-770 and of R334W-CFTR by VX-770, both in nasal cells and cell lines. However, these modulators fail to rescue H1079P or R347P. Data from organoids showed that D1152H, D614G, 3849+10Kb C>T were rescued by VX-770 alone and D614G, R334W, P205S and 2789+5 G>A were rescued by VX-809/VX-770 or VX-661/VX-770 combinations. We also identified distinct levels of response to CFTR modulators among patients with the same genotype. Altogether our results show that characterization of rare CFTR mutants can be best determined by combined data from different patient samples and cellular models towards a personalized therapy approach.

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Drug repurposing for cystic fibrosis subjects with rare *CFTR* mutations

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The first-generation of clinically available CFTR-correcting compounds, ivacaftor (KALYDECO®, VX-770) and ORKAMBI® have elicited significant clinical improvements for people suffering from cystic fibrosis (CF) but have only been available to CF subjects harbouring 34 out of the more than 2000 reported *CFTR* mutations. Clearly, a need for new CFTR-modulating therapies for CF subjects with other (rare) *CFTR* mutations exists.

We have started the RAINBOW project in which we will screen >1400 FDA-approved drug compounds (including ivacaftor, lumacaftor and ORKAMBI®) in intestinal organoids derived from a total of 150 Dutch CF patients carrying rare uncharacterized *CFTR* mutations (with a prevalence of < 0.5% in the Dutch CF population), which include missense, frameshift, insertion/deletion, nonsense, and splicing mutations.

First, cytotoxicity of the FDA-approved drug compounds was assessed in heterozygous F508del/S1251N and homozygous F508del organoid cultures by performing a live-dead assay, assessing the intestinal organoid morphology and by determining the organoid swelling capacity upon forskolin induction after compound treatment. Based on these read-outs, 31 compounds (2.1% of the total library) were excluded from the FDA-approved drug library as these were too toxic to the organoids.

Next, we performed a primary screen, so far in 85 organoid cultures, by using our high-content 384-well forskolin-induced swelling assay (HTS-FIS) and identified several compounds capable of (partially) restoring CFTR function. To determine the optimal dosage and incubation time(s) of these compounds, we performed a secondary screen in parallel for further validation of these hits by using the conventional FIS assay (96-well screening format) and our recently developed steady-state lumen area (SLA) assay.

Altogether, our results show the potential of identifying new CFTR modulating compounds for CF subjects with rare, uncharacterized *CFTR* mutations by repurposing existing FDA-approved drug compounds.

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A collection of intestinal epithelial organoids to support the development of drugs and diagnostics in Cystic Fibrosis by combining CFTR functional tests in personalized medicine

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Background: *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. A recent development in the field is the expansion of intestinal stem cells into closed organoids containing crypt-like structures and an internal lumen lined by differentiated cells, recapitulating the in vivo tissue architecture.

Methods: We set the necessary infrastructure for collecting, growing and analyzing by forskolin induced swelling (FIS) assay) intestinal organoids from CF and non-CF cases. Organoids were established from 12 of 14 non-CF, 19 of 27 CF, and 7 from 8 CRD (CFTR related disease) subjects; in most of the cases n>3 vials have been stored. FIS assays performed after thawing provided overlapping results with fresh samples. FIS assay was performed before and after exposure to VX809, VX770 and PTC124. Organoids were obtained from cells recovered after performing Intestinal current measurement (ICM) as well as from fresh biopsies.

Results: In non-CF organoids swelling was completely blocked by the CFTR (inh)-172 and significantly enhanced following treatment with the potentiator 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 PTC124, but not in its absence. In this patient improvement of lung function (FEV1) was detected 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. We tested 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. We observed for the three F508del/F508del patients a very low basal organoid swelling correlated with the expected values of ICM for PI-CF patients (Derichs et al., Thorax 2010) and SCC in the CF range of values (>60 mmol/L).

Conversely, a PS-CF patient F508del/D1152H presented a basal organoid swelling in the range of non-CF subjects, ICM values in PS-range and SCC values of 31mmol/L.

Of note is the case of a CF patient F508del/2789+5G>A, that demonstrated a basal organoid swelling consistent with a ICM value in the CF range (-3,5 µA/cm2) and a high SCC (101 mmol/L).

Conclusions: This study reinforces the need to personalize therapy in CF, confirming intestinal organoids as a suitable tool for this purpose. All our data, taken together, suggest that organoid swelling could discriminate between individuals with different diagnosis, different CFTR genotypes and, being correlated with the well established CFTR biomarkers ICM and SCC. Comparison with established CFTR biomarkers aims to provide tools for individualized therapies.

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Limited rescue of W1282X-CFTR function by CFTR-restoring compounds in organoids

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Approximately 2,000 different mutations in the cystic fibrosis transmembrane conductance regulator *(CFTR)* gene cause functional defects in the CFTR anion channel and are the underlying cause of cystic fibrosis (CF). Around 10% of these mutations result in the formation of premature termination codons (PTCs) and the synthesis of truncated and nonfunctional CFTR proteins. No therapies to suppress class I *CFTR* nonsense mutations are currently available to individuals harboring these mutations despite previous and ongoing efforts in the field.

In our laboratory we developed a patient-specific CFTR function readout in rectal organoids, the forskolin-induced swelling (FIS) assay, which allows for measurement of residual CFTR function and response-to-therapy by quantification of fluid secretion. Importantly, these assays have successfully predicted responders to CFTR modulators and the lack of PTC read-through agent efficacy in organoid cultures of individuals with compound heterozygous nonsense mutations.

Until now, only two approved CFTR modulators, VX-770/KALYDECO® and VX-770/VX-809 (ORKAMBI®), have been registered for around 40 *CFTR* mutations but not for any class I mutations. A case study has been published, however, in which modest clinical benefit was reported for a patient with homozygous W1282X-CFTR mutations upon KALYDECO® treatment. Here, we present results of limited functional read-through in homozygous W1282X-CFTR (c.3846G>A, p.Trp1282X) organoids by VX-770/VX-809. An increase in organoid swelling was observed in homozygous W1282X-CFTR organoids but this phenotype was absent or reduced in homozygous G542X-CFTR (c.1624G>T, p.Gly542X) organoid cultures. These data suggest that individuals with W1282X-CFTR mutations might benefit from existing available CFTR modulators and potential future CFTR-restoring compounds, e.g. tezacaftor (VX-661).

Additionally, we will present preliminary data of a high-content FIS screen (HCS-FIS) performed in homozygous W1282X-CFTR organoids treated with around 1,400 (clinically available) FDA-approved drug compounds in combination with existing CFTR modulators to identify potential compounds that might enhance the VX-770/VX-809-rescued W1282X-CFTR function in these organoids harboring nonsense mutations. These data confirm and extend observations from others that late PTCs lead to production of functionally-rescuable CFTR protein.

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Effects of lumacaftor-ivacaftor therapy on CFTR function in Phe508del homozygous patients with Cystic Fibrosis

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The combination of the CFTR corrector lumacaftor with the potentiator ivacaftor has recently been approved for the treatment of patients with cystic fibrosis (CF) homozygous for the Phe508del CFTR mutation. The pivotal phase 3 trials examined clinical outcomes, but did not evaluate CFTR function in patients. Hence, we wanted to examine the effect of lumacaftor-ivacaftor on biomarkers of CFTR function in Phe508del homozygous CF patients aged 12 years and older.

This prospective observational study assessed clinical outcomes including FEV1 % predicted and BMI, and CFTR biomarkers including sweat chloride concentration, nasal potential difference (NPD) and intestinal current measurement (ICM) before and 8-16 weeks after initiation of lumacaftor-ivacaftor therapy.

A total of 53 patients were enrolled in the study and 52 patients had baseline and follow up measurements. After initiation of lumacaftor-ivacaftor, sweat chloride concentrations were reduced by 18 mmol/L. Further, NPD and ICM showed partial rescue of CFTR function in nasal and rectal epithelia to levels of 10% and 18% of normal, respectively. All patients improved in at least one CFTR biomarker, but no correlations were found between CFTR biomarker responses and clinical outcomes.

We could show that lumacaftor-ivacaftor results in partial rescue of Phe508del CFTR function to levels comparable to the lower range of CFTR activity found in patients with residual function mutations. Functional improvement was detected even in the absence of short-term improvement of FEV1 % predicted and BMI.

P120

Ivacaftor potentiation of CFTR in healthy human adults: single gland sweat rate analysis

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Purpose: To determine if oral ivacaftor (Kalydeco®, VX-770) influences human wild type CFTR function in vivo, we measured CFTR-dependent (C-sweat) and CFTR-independent (M-sweat) rates from multiple identified sweat glands in 8 healthy subjects (including 3 F508del carriers).

Methods: The two types of sweating were stimulated sequentially with intradermal injections of appropriate reagents: methacholine for M-sweat followed by a cAMP-elevating cocktail of isoproterenol and aminophylline, plus atropine to block M-sweat. Each gland served as its own control via alternating off-drug on-drug tests on both arms. Tests occurred at weekly intervals with 3 off and 3 on tests per subject; M-sweating was measured for 10 min and C-sweating for 30 min, with imaging at 30 sec intervals.

We varied the concentration of the β-adrenergic cocktail. A full cocktail was formulated to stimulate CFTR maximally and a 1% concentration (with same level of atropine) only partially. All tests used the same concentration of methacholine to stimulate M-sweating. For each subject we optically measured ~700 sweat bubble volumes in an oil layer on the arm (43-112 glands per arm, maximum 12 readings per gland). We log-transformed the readings and applied linear mixed models to the data from each subject, and to the pooled data. These models allow for the inclusion of all glands, unlike paired t-tests, which were also run.

Results: Mixed models analysis of pooled data show that ivacaftor increased log-volume of C-sweat stimulated by the 1% cocktail condition by 16.45 ± 2.25%, ($p = 6.86e-8$, n=5 subjects, 762 glands); C-sweat stimulated by full cocktail by 6.6 ± 3% ($p = 0.035$, n= 5 subjects, 695 glands), and M-sweat by 2.14 ± 2.23% ($p = 0.34$, n = 8 subjects, 1457 glands). With paired t-tests (using the strict significance level, $P < 0.001$ to control the false positive rate due to multiple comparisons) ivacaftor significantly increased C-sweat in all 5 subjects tested with 1% cocktail and 3/5 subjects with full cocktail. For M-sweat, 6/8 subjects showed no significant change, one significantly increased and one decreased.

Concurrent sweat chloride tests (3 off and 3 on for each of 6 subjects and 6 off/on for one subject) detected no effect of ivacaftor; averages off-drug = 25.1 ± 11.4 vs. on-drug 24.9 ± 6.1 ($p = 0.9$).

Conclusion: We conclude that ivacaftor in vivo increases the open channel probability of wild type CFTR, provided it is not already maximally stimulated, and that he single gland sweat rate method was sensitive enough to detect the changes. The inability to detect a change in sweat chloride values may reflect the maximally activated state of CFTR in the sweat duct, as well as the decreasing sensitivity of sweat chloride measurements as CFTR function increases. Supported by an Investigator Initiated Study Grant from Vertex Pharmaceuticals.

Clinical Trial Number: NCT02310789

P121
CFTR protein as a predictive biomarker

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Cystic fibrosis (CF) patients homozygous for the F508del mutation now have therapeutic options with approved CFTR modulator, Orkambi, but the clinical experiences have shown a highly variable patient response to Orkambi. Because CFTR correctors and potentiators demonstrate substrate-limited efficacy in the clinic, we hypothesize that CFTR expressions may be predictive of the response to Orkambi, a commercially available product composed of ivacaftor, a potentiator, and lumacaftor, a corrector. Human nasal epithelial (HNE) cultures were generated from 16 CF donors homozygous for F508del. Initial characterization of the HNE cultures included CFTR activity by Ussing chamber and CFTR expression by qPCR and ELISA. The average basal forskolin-induced current was 1.19+0.44 μAmps/cm^2 across the 16 donors. Inter-donor variability for CFTR mRNA was large and showed almost a 10x-fold range in values compared to only a 2x-fold range in CFTR protein levels. Interestingly, CFTR mRNA and protein positively correlated with each other but both showed only modest correlations with basal CFTR activity. HNE-CF cultures treated with lumacaftor showed an average increase of 1.00 μAmps/cm^2 in forskolin-induced current. Basal current positively correlated with the final measured forskolin-induced current but not the absolute change in current from basal activity. Both CFTR mRNA and protein also positively correlated with forskolin-induced current as well as the absolute change in current following lumacaftor treatment. These findings were also observed in cultures treated with lumacaftor followed by acute ivacaftor treatment. The data suggests that CFTR mRNA and protein levels are predictive of increases in CFTR activity in nasal epithelial primary cultures treated with lumacaftor+ivacaftor. Our objective is to confirm the observed correlation in biological samples from CF patients for whom lung function data before and after Orkambi therapy is available. If confirmed, measurement of CFTR mRNA and protein levels in F508del homozygous subjects may be implemented as a predictive biomarker to identify Orkambi responders.

P122

Characterization of novel urinary exosomal biomarkers for monitoring the effects of CF pathophysiology in patients

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Current strategies designed to measure the effectiveness of treatments counteracting the pathological effects of Cystic Fibrosis (CF) require invasive medical and clinical procedures and lack sensitivity to detect relevant physiological changes reflecting the progression of the disease. To better characterize the effects of these therapeutics in CF patients, we proposed to identify novel CF protein biomarkers transported in exosomes secreted into physiological fluids in order to accurately measure treatment efficacy monitoring changes in the treated CF patient exosomal proteomic profile.

Exosomes are small extracellular vesicles (EVs) budding inward off the membrane of multi-vesicular bodies (MVB) encapsulating cytosolic contents (reviewed in Kowal et al, 2014). These vesicles transport soluble and membrane-bound proteins also trafficked through other secretory, recycling and degradation pathways and therefore may carry biomarkers reflecting the state of cellular homeostasis in general and the quality of misfolded CFTR intracellular trafficking and activity at the plasma membrane (PM) in CF cells in particular. Upon MVB fusion with the PM, exosomes are secreted into the extracellular space and may be directed to easily and non-invasively sampled physiological fluids from which they are routinely isolated and characterized using very well described procedures.

Here, we specifically investigated CF biomarkers trafficked in exosomes isolated in urine sampled from age-matched healthy controls, and non-treated and Orkambi-treated deltaF508 homozygote CF patients. We successfully isolated and enriched urinary exosomes by differential centrifugation and density gradient fractionation evidenced by the presence of the strong exosomal markers CD63, CD81, syntenin and TSG101 detected by Western blotting and immuno-electron microscopy techniques in our EV fractions.

Our preliminary differential proteomic analysis comparing cargo composition of deltaF508 homozygote CF patient and healthy control urinary exosomes by mass spectrometry identified about 1300 proteins across all samples with higher levels for several lysosomal proteins in CF samples and higher levels for Rab-27A, a regulator of exosome secretion, in controls. These results show that the CF pathobiology modifies urinary exosome proteic composition and suggest exosomal cargo origin, loading or maturation differential regulation in CF cells.

We are using the same strategy to read the proteomic profile of exosomes isolated from urine samples of Orkambi treated deltaF508 homozygote patients to characterize local treatment effects on CFTR expressing renal epithelial cells and other systemic effects of the drugs in other tissues, such as the respiratory tracts, carried in the urine.

In conclusion, the characterization of novel CF-specific biomarkers in exosomes isolated from physiological fluids constitutes a non-invasive tool to diagnose the disease and assess CF progression in patients. In addition, monitoring levels of these biomarkers in exosomes isolated from treated CF patients can potentially measure treatment effectiveness to correct CFTR addressing and activity and represents a new method of testing and selecting most effective personalized therapeutic strategies.

P123

Follow-up of CF patients using DNA methylation markers and sputum samples

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During the last decades, the quality of life of CF patients as well as their life expectancy have greatly increased mainly because of an improved follow-up. Currently, many clinical parameters, namely the FEV1, and a few molecular markers are used to evaluate lung disease severity in CF, but, no genomic nor epigenomic markers have been developed for the follow-up of CF patients. By combining high-throughput technologies with big data analysis, we aim to develop and validate DNA methylation prognostic markers that correlate with the severity of lung disease and with the evolution of the lung function.

In the discovery step, using genome-wide DNA methylation data generated during a previous study, we identified potential markers. DNA methylation was assessed using the 450K BeadChip array on nasal epithelial cell samples of the METHYLCF cohort (Magalhães *et al*., 2017). We have selected 30 CpG dinucleotides whose methylation levels correlate with FEV1 (potential markers of lung disease severity), and 30 CpG that correlate with FEV1 variations (potential markers of the evolution of the lung function).

In the confirmatory step, we are assessing 12 potential epigenetic markers in sputum samples collected from MethylBiomark, an independent cohort under construction (90% of patients included). This longitudinal cohort consists of 50 CF patients, aged 12-30y and carrying two severe *CFTR* mutations in *trans*. Clinical data are recorded and sputum samples are collected at the baseline visit and every six months during the 1.5-year follow-up. DNA methylation markers are assessed by pyrosequencing, a fast and cost-effective technique that is already used for routine clinical testing.

The specific objective of this project is to assess whether changes in DNA methylation allow us to predict lung disease evolution in CF patients. The final long-term goal is to develop prediction models to guide healthcare professionals in their decision-making process regarding CF patients management.

P124

LncRNAs: emerging players in *CFTR* gene regulation

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Objectives: *CFTR* gene displays a tightly tissue specific and temporal expression pattern that still remain incompletely understood. Recently, we showed that transcription factors and small non-coding RNAs (miRNAs) act in synchrony to explain the weak *CFTR* mRNA level in mature lung cells. By now, the involvement of other key regulatory players, the long non-coding RNAs (lncRNAs), has been investigated in the control of genes showing tissue-specific expression. These lncRNAs can operate locally near their sites of synthesis. In this work, as we identified the presence of nine lncRNAs within the *CFTR* locus, we decided to define their role on the *CFTR* gene expression.

Materials and Methods: Expression analysis by RT-qPCR has been conducted in 20 different human tissues from several donors and in cell lines (BEAS-2B, 16HBE14o-, T84). For CF003 and CF006 lncRNAs, antisense oligonucleotides (GapmeRs) were designed to inhibit their expression and expression vectors for overexpression assays were constructed. Gapmers and expression vectors have been transfected in bronchial cells and lncRNAs or *CFTR* mRNA level were assessed by RT-qPCR. Luciferase reporter assays were carried out in BEAS-2B by co-transfection of lncRNAs and vectors containing different length of *CFTR* promoter.

Results: Examination of the *CFTR* locus and adjacent regulatory regions by using databases collecting human annotated lncRNAs led to the identification of 9 lncRNAs lying in the *CFTR* locus harboring signatures of active transcription as polyadenylation signals, and well-conserved canonic splicing sites. Quantitative analysis showed a detectable expression of 6 non-coding RNAs (Ct from 27 to 32) underlying their biological existence. Interestingly, these lncRNAs display a tissue-specific expression profile. First of all, we focused on CF003 and CF006, which are overexpressed in nasal cells taken from CF patients. To assess whether these lncRNAs could exert *cis*-acting role on *CFTR*, we used Gapmers to inhibit their expression. RNA silencing of CF003 and CF006 induced a two-fold increase in *CFTR* mRNA level in BEAS-2B cells, and conversely, their overexpression led to a significant decrease of *CFTR* transcripts level. Interestingly, opposite results were observed in 16HBE14o-, a model with a marked *CFTR* expression. These data suggest their implication in the control of *CFTR* gene expression in cells dependently on the *CFTR* level. By using *in silico* tools, we predicted binding sites for these lncRNAs on *CFTR* promoter. By luciferase reporter assays with different length of *CFTR* promoter, we showed that CF003 and CF006 induced an up-regulation of luciferase under the control of the 1kb-*CFTR* promoter. To confirm whether these lncRNAs act directly on *CFTR* promoter, mutagenesis of their binding sites are on progress. Characterization of their cellular localization and their transcriptional unit are still on progress.

Conclusion: In this study, we characterized new lncRNAs transcribed in the *CFTR* locus exhibiting specific tissue-expression profiles. Our data highlight the role of new transcriptional regulators of *CFTR* gene, the lncRNAs CF003 and CF006. These new partners could represent promising targets for future therapeutic in CF.

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P125
Complex network involving miRNA and RNA-BP in CF

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Background: Cystic fibrosis (CF), the most common lethal rare disease, is characterized by lung inflammation. Changes in miRNAs expression profile in CF cells have been described in the literature, mainly by quantification of a part of the miRNA pool (TLDA system).

Aims: In order to obtain an overview, we decided to use an unbiased sequencing approach (Illumina) to establish profiling of miRNA expression. First, we defined the genes and/or signalling pathways targeted by these deregulated miRNAs. Secondly, we focused on an anti-inflammatory protein, named TTP and studied its regulation and impact on its targets mRNAs.

Materials and methods: RNAs or proteins were extracted from CF bronchial cell lines or from *ex vivo* reconstituted epithelium cultured in Air-Liquid Interface, ALI (5 non-CF and 5 CF). To assess miRNAs and TTP role, expression level of miRNAs, transcripts and proteins of interest was evaluated by RT-qPCR or immunoblot, respectively. To confirm TTP binding on *CFTR*-3'UTR RNA pull-down assays were also carried out.

Results: In CF ALI cultures, we showed the deregulation of 35 miRNAs, which mainly target mRNAs involved in PI3K-Akt pathway (21/35) and MAPK pathway (18/35). To validate the role of these genes in CF, we quantified the expression of more than 200 genes by qPCR and specific arrays, and confirmed the deregulation of more than 30 genes. In addition, search for a crosstalk between PI3K-Akt and MAPK pathways pointed out RNA-BPs that control gene expression of several genes. As a good candidate, we next focused on the TTP protein, a RNA-BP targeted by 6 deregulated miRNAs and deregulated in CF cells. The TTP non-phosphorylated form modulates negatively the stability of many interacting partners including pro-inflammatory proteins, such as IL-8. We showed by immunoblot an accumulation of both the TTP phosphorylated form in CF cells (inactive form) and the MK2 phosphorylated form, responsible for TTP inactivation. By using drugs we showed that MK2 protein, typically activated through MAPK pathway, is phosphorylated by Erk pathway in CF cells. Next, we searched for TTP's targets and *in silico* analysis (RBPDB database) predicted the presence of binding sites on the 3'UTR of *CFTR* mRNA. Occupancy of TTP on *CFTR* mRNA was validated by RNA pull-down. Overexpression and silencing of TTP showed a positive effect on *CFTR* mRNA. Impact on other targets are being tested.

Conclusion: miRNAs studies allowed us to highlight a new regulation pathway in CF cells, involving an anti-inflammatory protein. Decoding miRNAs/TTP partners' map could be of great interest in CF.

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Identification of SLC26A9 chloride channel activators by high-throughput screening

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Introduction & Aims: The SLC26A9 chloride channel represents a promising candidate to provide apical chloride transport in the absence of functional CFTR, thus circumvent the primary defect in cystic fibrosis. Recent evidence suggests that SLC26A9 Cl^- channel function may be activated therapeutically by compounds that increase translocation of the protein into the apical plasma membrane. To systematically identify therapeutic target genes and lead compounds promoting trafficking of SLC26A9, we aim to perform high-throughput siRNA and chemical library screens.

Methods: We generated CFBE41o- cells with stable expression of HA-tagged SLC26A9. To be able to measure chloride channel function on a high-throughput scale, we developed a live-cell microscopy-based assay using a membrane potential sensitive dye (FLIPR). Following siRNA-mediated knockdown, changes in membrane potential upon chloride channel inhibition by niflumic acid are measured by time-lapse imaging in 96-well format. Intensity time-traces for individual cells are quantified after segmentation and image quality control. Validation of the effect of siRNA silencing on chloride current is tested in Ussing chamber.

Results: Using the FLIPR assay we quantify the baseline and the response upon adding inhibitor. We were able to detect significant differences between fluorescence intensity changes of scrambled and SLC26A9 siRNA silenced cells. Transepithelial short-circuit current measurements showed that SLC26A9 knockdown significantly reduced the basal and the cAMP-stimulated chloride current, as well as it decreased the inhibition by niflumic acid.

Conclusions/Perspectives: We have developed a robust high-throughput assay to monitor SLC26A9 function. Currently we are testing a hypothesis driven siRNA library for SLC26A9 modulator genes. Hits from a primary screen can be readily validated by Ussing chamber. This platform will enable us in the future to identify therapeutic strategies to activate SLC26A9.

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Characterization of airway and bone marrow derived macrophages in a mouse model of cystic fibrosis

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Introduction: Airway macrophages play a major role in the first line of defense of the innate immune system of the lungs. In cystic fibrosis (CF) this function is even more important since there is a chronic and excessive airway inflammation triggered by neutrophils. During the last years, an increasing number of publications gave indications that mutations in the *cystic fibrosis transmembrane conductance regulator* (*CFTR*) gene not only cause malfunctions of the Cl^- and bicarbonate CFTR channel in epithelial cells but in macrophages as well (Bruscia & Bonfield; J Innate Immun; 2016). In our project we investigated effects of bone marrow transplantation in CF mice and characterized engrafted cells in the lungs of mouse chimeras. Furthermore, we analyzed *in vitro* bone marrow derived macrophages regarding their lysosomal acidification.

Methods: Transplantation of hematopoietic stem cells (HSPCs) from wild type mice in a CF mouse model revealed a beneficial effect in these chimeric CF mice (CF^{B6}) under infectious conditions. Infecting mice with *P. aeruginosa*, the key bacterial pathogen for CF lung disease, we monitored a milder course of the infection, reduced lung bacterial numbers and last but not least increased survival in CF^{B6} mice compared to CF mice transplanted with isogenic CF stem cells (CF^{CF}). Based on these results, we tracked transplanted cells in CF^{B6} mice via the leucocyte surface antigen CD45, since recipient CF and wild type donor mice are carrying the different alleles CD45.1 and CD45.2, respectively. Flow cytometry analysis of macrophages from the lungs of chimeric mice showed an almost complete replacement of the original CF macrophages by the transplanted CD45.1 cells, which engrafted successfully in the lungs. These data were enhanced by immunofluorescence of the lungs of chimeric mice. Transplanted CD45.1 cells could be detected and stained with the typical macrophage marker CD68. In parallel, we set up an assay to evaluate differences in the pH of bone marrow differentiated macrophages (BMDM) of CF and wild type mice. In these macrophage-like cells we measured fluorescence with the pH-sensitive dye LysoSensor Green DND-189 via confocal microscopy (Zhang et al. J Immunol. 2010). Our results displayed a significant less acidic pH in CF-BMDM confirming the hypothesis of an impaired acidification in lysosomes of CF macrophages compared to healthy ones.

Conclusions: In summary, our results strengthen the hypothesis of Cftr holding an important role not only in epithelial cells, but in phagocytes as well. Since we found the majority of lung macrophages possessing the donor CD45.1 allele in our transplantation model, we conclude that the introduced healthy *Cftr* gene is responsible for the improved immune answer of the chimeric mice under *P. aeruginosa* infection. As a next step, we are going to investigate macrophages of CF mice transplanted with wild type hematopoietic stem cells to find out if this approach will restore malacidification in the airway macrophages and might be of therapeutic interest for CF patients to combat their chronic lung infections.