



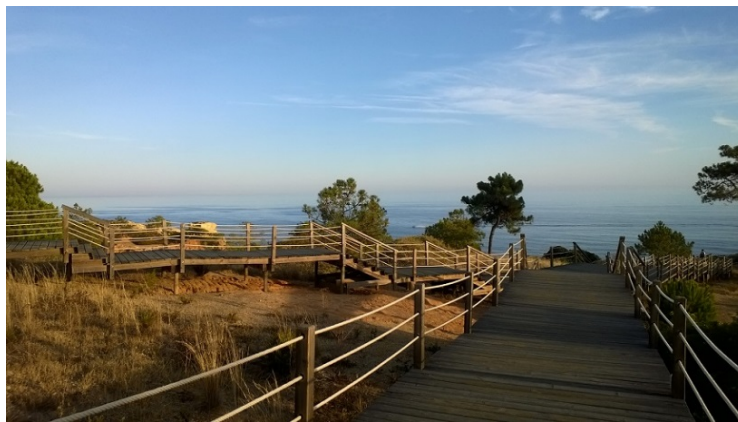
2015

European Cystic Fibrosis Society

12th ECFS Basic Science Conference

Conference Programme & Abstract Book

Albufeira, Portugal



Chairpersons

Marc Chanson, Frédéric Becq and Martina Gentzsch

25 March – 28 March 2015

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CONFERENCE SPONSORSHIP & SUPPORT



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italian cystic fibrosis research foundation



WELCOME FROM THE ECFS PRESIDENTS

Dear Friends and Colleagues,

It is a great pleasure to welcome you in Portugal to the 12th European Cystic Fibrosis Conference entirely dedicated to Basic Science.

This year we are delighted to welcome Dr. Marc Chanson (CH) who will be supported by Prof. Frédéric Becq (FR) and Dr. Martina Gentsch (US).

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 I hope productive collaborations.

We extend a very warm welcome to an exciting conference.



Stuart Elborn
ECFS President



Kris De Boeck
ECFS President Elect

CONFERENCE CHAIRPERSONS' WELCOME

Dear Friends and Colleagues,

Located in the south of Portugal, in the centre of the Algarve, Albufeira is an exceptional place to discover a landscape full of contrasts.

Albufeira is one of the oldest cities in the Algarve. Its name derives from Arabic and means "Sea Castle", probably due to the proximity of the village to the sea or lagoon that was formed in the lower part of the area.

In older times it was a fishing village and artists, painters, poets and writers fell in love with it. Albufeira has become one of the most popular and visited tourist centres in the South of Europe.

It is said that it was here, while looking at the intensity of the blue sea that Paul McCartney wrote "Yesterday", one of the most beautiful songs by the Beatles.

But let's come back to today and to the exciting next days!

With this conference in Albufeira, you can expect to hear about recent unpublished results and engage in 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 and vivid discussions. 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, one debate, as well as a Flash Paper session.

This combination has been extremely successful in the past and the conference offers a forum for informal interactive discussions conducive to brain storming to generate new ideas. On Thursday and Friday evening we will finish the day with poster sessions. And a novelty this year: all delegates will have the opportunity to vote for best poster prize!

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.



Marc Chanson
University of Geneva
Switzerland



Frédéric Becq
University of Poitiers
France



Martina Gentsch
University of North Carolina at
Chapel Hill
United States

**2015 ECFS Conference
New Frontiers in Basic Science of Cystic Fibrosis**

25 March- 28 March 2015, Albufeira, Portugal

Programme

Chairpersons: Marc Chanson (Geneva, Switzerland)
Frédéric Becq (Poitiers, France), Martina Gentzsch (Chapel Hill, NC, United States)

Wednesday, 25 March 2015 (Day 1)

09:30-12:00 **ECFS Cystic Fibrosis Molecular & Cell Biology and Physiology Basic Science Working Group Meeting**

12:00-14:00 *Light lunch*

14:00-17:00 **Pre-Conference Seminar - CFTR functional repair: towards suitable systems for better drug screening**

Coffee break – 15:30

17:30-18:00 **Official Opening of the Meeting by the Conference Chairpersons**

18:00-19:00 **Opening Keynote Lecture**

Protective HCO_3^-

Paul M. Quinton (US)

19:00-19:45 **Welcome Reception**

19:45-21:30 *Dinner*

Thursday, 26 March 2015 (Day 2)

07:30-08:45 *Breakfast*

08:45-10:30 Symposium 1 – CFTR folding and structure

Chairs: Christine Bear (CA) – Nikolay Dokholyan (US)

08:45-09:10 CFTR purification and advances towards a high resolution structure – Bob Ford (UK)

09:10-09:35 Towards an understanding of NBD1 and NBD2 heterodimerization – Andrew Chong (CA)

09:35-10:00 ABCs in Adaptive Immunity and Viral Immune Evasion – Robert Tampé (DE)

10:00-10:10 Abstract 04 - Motions of CFTR membrane spanning helices related to channel gating - Nikolay Dokholyan (US)

10:10-10:20 Abstract 01 - Molecular Dynamics simulations on the wild-type and F508del CFTR 3D models - Brice Hoffmann (FR)

10:20-10:30 Abstract 02 - Stability of CFTR and the effects of Cystic fibrosis-causing mutations in humans - Xin Meng (UK)

10:30-11:00	<i>Coffee break & Poster viewing</i>
11:00-12:45	Symposium 2 – Regulation of CFTR expression and activity Chairs: Jean-Michel Sallenave (FR) – Alan Verkman (US)
11:00-11:25	Revertant mutants modify, but do not rescue, the gating defect of the cystic fibrosis mutant G551D-CFTR - David Sheppard (UK)
11:25-11:50	The CFTR channel as an allosteric protein: bypassing the normal requirements for ATP binding and R domain phosphorylation – Kevin Kirk (US)
11:50-12:15	Structural dynamics of the CFTR pore opening conformational transition - László Csanády (HU)
12:15-12:25	Abstract 09 - Anoctamins control epithelial Cl ⁻ secretion by CFTR - Inês Cabrita - Regensburg (DE)
12:25-12:35	Abstract 23 - Role of miRNAs in the CF pathology - Jennifer Bonini (FR)
12:35-12:45	Abstract 06 - EPAC1 activation by high levels of cAMP stabilizes CFTR at the membrane through interaction with NHERF-1 - Carlos Farinha (PT)
12:45-14:30	<i>Lunch</i>
14:30-15:30	Flash Poster Session (even numbers) Chair: Anil Mehta (UK)
15:30-16:00	<i>Coffee break & Poster viewing</i>
16:00-17:45	Symposium 3 – CFTR and bicarbonate in pancreas inflammation Chairs: Mike Gray (UK) – David Sheppard (UK)
16:00-16:25	Involvement of CFTR in glucose-induced electrical activities and insulin secretion in pancreatic islet β -cells – Hsiao Chang Chan (CN)
16:25-16:50	CFTR inhibition and elevated mucus concentration are key events in pancreatitis - Peter Hegyi (HU)
16:50-17:15	CFTR and bicarbonate in CF disease - David Withcomb (US)
17:15-17:25	Abstract 21 - Orphan G-protein coupled receptor CELSR3 is an epithelial marker downregulated in cystic fibrosis - Sabrina Noël (BE)
17:25–17:35	Abstract 45 - Imbalance of mucus homeostasis in chronic pancreatitis - Anita Balázs (HU)
17:35-17:45	Abstract 25 - Differential gene activation in normal and cystic fibrosis human bronchial epithelial cells upon <i>Pseudomonas aeruginosa</i> infection - Loïc Guillot (FR)
17:45-18:00	Break
18:00-19:00	Flash Paper Session Chair: Robert Ford (UK)
18:00-18:15	FP01: Transcription factors and miRNAs that regulate fetal to adult CFTR expression change are new targets for cystic fibrosis - Magali Taulan-Cadars (FR)
18:15-18:30	FP02: MiR-31 Dysregulation in Cystic Fibrosis Airways Contributes to Increased Pulmonary Cathepsin S Production - Sinéad Weldon (UK)
18:30-18:45	FP03: Impaired TLR4 and HIF expression in cystic fibrosis bronchial epithelial cells downregulates hemoxygenase-1 and alters iron homeostasis in vitro - Shashi Chillappagari (DE)
18:45-19:00	FP04: Restoration of CFTR function in patients with cystic fibrosis carrying the CFTR F508del mutation - Luigi Maiuri (IT)
19:30-21:30	<i>Dinner</i>
21:30-23:00	Evening Poster Session: Posters with Even numbers

Friday, 27 March 2015 (Day 3)

07:30-08:45 *Breakfast*

08:45-10:30 **Symposium 4 – Host-pathogen interaction** **Chairs: Jeffrey Beekman (NL) – David Stoltz (US)**

08:45-09:10 Modulation of the host immunity by *Pseudomonas aeruginosa* and *Staphylococcus aureus*: impact on the pulmonary microbiome - Lhousseine Touqui (FR)

09:10-09:35 Host-pathogen interactions in CF lung disease - Dominik Hartl (DE)

09:35-10:00 Normalisation of inflammatory responses in CF through induction of the NF-kappaB regulator A20 - Bettina C. Schock (UK)

10:00-10:10 Abstract 27 - Dissection of host susceptibility to *Pseudomonas aeruginosa* lung infection in mice - Maura De Simone (IT)

10:10-10:20 Abstract 35 - A new synthetic compound inhibits the expression of Quorum-Sensing regulated genes and biofilm formation in *Pseudomonas aeruginosa* - Aurelie Furiga Chusseau (FR)

10:20-10:30 Abstract 29 - *Pseudomonas aeruginosa* adaptation to cystic fibrosis airways shapes the host response in mice during the progression of airway disease - Camilla Riva (IT)

10:30-11:00 *Coffee break & Poster viewing*

11:00-12:45 **Symposium 5 - Mucus in lung health** **Chairs: Marcus Mall (DE) – Paul M. Quinton (US)**

11:00-11:25 Mucociliary Interactions in Health and Disease - Brian Button (US)

11:25-11:50 The respiratory mucus barrier; structure, assembly and intracellular packaging of the major gel-forming mucin, MUC5B - Dave Thornton (UK)

11:50-12:15 Mucociliary Transport in Early CF - David Stoltz (US)

12:15-12:25 Abstract 20 - Defective PPAR γ signaling and increased mucin/bicarbonate expression in the regulation of human airway epithelial cell repair - Joanna Bou Saab (CH)

12:25-12:35 Abstract 46 -Structure activity relationship for OligoG-induced normalization of the CF mucus phenotype - Anna Ermund (SE)

12:35-12:45 Abstract 29 - The role of mucus and mucin in mediating *Pseudomonas aeruginosa* infection and virulence in cystic fibrosis - Patrick Moore (IE)

12:45-14:00 *Lunch*

14:00-18:30 *Free Afternoon*

18:30-19:30 **Flash Poster Session (odd numbers)**
Chair: Mohamed Benharouga (FR)

19:30-21:30 *Dinner*

21:30-23:00 **Evening Poster Session: Posters with Odd numbers**

Saturday, 28 March 2015 (Day 4)

07:30-08:45 Breakfast

08:45-10:30 Symposium 6 – CFTR modulators and pharmacological correction of cystic fibrosis

Chairs: Luis Galletta (IT) – Mitch Drumm (US)

08:45-09:10 Small molecule screens for CFTR modulators – Alan S. Verkman (US)

09:10-09:35 CFTR modulators for treatment of Cystic Fibrosis – Katja Conrath (BE)

09:35-10:00 Challenges in Pharmacological Correction of Mutant CFTR – Deborah M. Cholon (US)

10:00-10:10 Abstract 66 - Analysis of the chronic effects of lumacaftor and ivacaftor on F508del-CFTR single-channel gating and stability - Yiting Wang (UK)

10:10-10:20 Abstract 70 - CFTR modulators and pharmacological correction of cystic fibrosis - Nicoletta Pedemonte (IT)

10:20-10:30 Abstract 47 - Mechanism-based screens lead to the identification of complementary pharmacological correctors of F508del CFTR - Feng Liang (US)

10:30-11:00 Coffee break & Poster viewing

11:00-12:15 Debate – Personalised Medicine for CF

Chair: Kris De Boeck (BE)

Faculty: Garry Cutting (US) – Harriet Corvol (FR) – Ian Balfour-Lynn (UK)

12:15-14:00 Lunch

14:00-15:45 Symposium 7 – Protease-antiprotease imbalance in CF

Chairs: Lhoussaine Touqui (FR) – Brian Button (US)

14:00-14:25 Elucidating in vivo roles of neutrophil elastase and other proteases in CF-like lung disease in mice – Marcus Mall (DE)

14:25-14:50 Role of Host and *Pseudomonas* proteases in the modulation of CFTR and innate immune responses – Jean-Michel Sallenave (FR)

14:50-15:15 Proteases in the Cystic Fibrosis Lung – elastase and its 'friends' – Cliff Taggart (UK)

15:15-15:25 Abstract 30 - Azithromycin effect on lung inflammation induced by *Pseudomonas aeruginosa* released proteases as shown by in vivo imaging in IL-8 transiently transgenized mice - Paola Melotti (IT)

15:25-15:35 Abstract 40 - Neutrophil Elastase-Cleaved SPLUNC1 Disrupts *Burkholderia Cepacia* Biofilms Better Than Intact SPLUNC1 - Robert Tarran (US)

15:35-15:45 Abstract 60 - Interactions of Cytokeratin 8 with misfolded Phe508del-CFTR and Z-alpha-1 antitrypsin – role in protein trafficking and/or degradation - Iwona Pranke (FR)

15:45-16:15 Coffee Break

16:15-17:45 Symposium 8 - CFTR trafficking

Chairs: Raymond Frizzel (US) – Aleksander Edelman (FR)

- 16:15-16:40 The role of the heterotrimeric G α 12 chaperone-like system in aberrant trafficking of CFTR channel - Mohamed Benharouga (FR)
- 16:40-17:05 CFTR regulation by VIP: molecular mechanism and therapeutic potential – Valerie Chappe (CA)
- 17:05-17:30 Identification of novel HSP70 dependent mechanisms for degradation of rare CFTR mutants – Scott A. Houck (US)
- 17:30-17:40 Abstract 14 - Inhibition of Hsp90 ATPase activity leads to correct F508del-CFTR defective trafficking in Cystic Fibrosis cells - Johanna Bertrand (FR)
- 17:40-17:50 Abstract 07 - Sustained increases in cytosolic Ca $^{2+}$ induce CFTR internalisation - Waseema Patel (UK)
- 17:50-18:00 Abstract 59 - Trafficking in CFTR Therapies - Dean Madden (US)

18:00-18:15 *Break*

18:15-19:15 Closing Keynote Lecture

Systems Approaches to Find Cystic Fibrosis Pathways and Therapies - Margarida Amaral (PT)

20:30 *Dinner / Social Event*

POSTER TITLES & AUTHORS

P1 Molecular dynamics simulations on the wild-type and F508del CFTR 3D models

Brice Hoffmann, Jean-Paul Mornon, Pierre Lehn, Isabelle Callebaut

P2 Stability of CFTR and the effects of Cystic fibrosis-causing mutations in humans

Xin Meng, Xiaomeng Wang, Naomi Pollock, Tracy Rimington, Robert Ford

P3 Towards an understanding of NBD1 and NBD2 heterodimerization

Andrew Chong, Robert Vernon, Rhea Hudson, Jennifer Dawson, Alaji Bah, Julie Forman-Kay

P4 Motions of CFTR membrane spanning helices related to channel gating

Jhuma Das, Andrey Aleksandrov, Liying Cui, Lihua He, John Riordan, Nikolay Dokholyan

P6 EPAC1 activation by high levels of cAMP stabilizes CFTR at the membrane through interaction with NHERF-1

Miguel Lobo, João Fernandes, Margarida Amaral, Manuela Zaccolo, Carlos Farinha

P7 Sustained increases in cytosolic Ca²⁺ induce CFTR internalisation

Waseema Patel, Abigail Marklew, Robert Tarran, Michael Gray

P8 Exploring the mechanisms behind cigarette smoke-induced internalization of the cystic fibrosis transmembrane conductance regulator

Abigail Marklew, Waseema Patel, Amanda Smith, Rodney Gilmore, Michael Gray, Robert Tarran

P9 Anoctamins control epithelial Cl⁻ secretion by CFTR

Inês Cabrita, Roberta Benedetto, Podchanart Wanitchakool, Karl Kunzelmann, Rainer Schreiber

P10 Multielectrode biosensor system to study ion fluxes across the monolayer of epithelial cells

Mirosław Zajac, Renata Toczyłowska-Maminska, A. Lewenstam, K. Dolowy

P11 Analysis of CFTR channel gating by hydrolysable ATP analogues with substitutions in the ribose sugar of ATP

Majid Al Salmani, Min Ju, Ming Yang, Yiting Wang, David Sheppard, Zhiwei Cai

P12 Safety and efficacy of short palate and lung nasal epithelial clone 1 (SPLUNC1)-derivatives for the treatment of CF lung disease

Dale Christensen, Shawn Terryah, Robert Fellner, Robert Taylor, Robert Tarran

P13 Iminosugars acting as mannosidase inhibitors in cystic fibrosis: design of multivalent correctors and identification of new therapeutically targets

Khadidja Sidelarbi, Johanna Bertrand, Frédéric Becq, Philippe Compain, Caroline Norez

P14 Inhibition of Hsp90 ATPase activity leads to correct F508del-CFTR defective trafficking in cystic fibrosis cells

Johanna Bertrand, Julien Henri, Virginie Cendret, Jérôme Désiré, Philippe Meyer, Yves Blériot, Frédéric Becq, Caroline Norez

P15 rAAV2/5 encoding truncated CFTR rescues CF phenotype in Δ F508 mouse model

Dragana Vidovic, Marianne Carlon, Melanie Faria da Cunha, Johanna Dekkers, Monika Hollenhorst, Marcel Bijvelds, Chris Van den Haute, Veerle Baekelandt, Hugo de Jonge, Isabelle Sermet-Gaudelus, Jeffrey Beekman, Aleksander Edelman, Rik Gijsbers, Zeger Debysen

P16 Characterization of the mutations identified in *cis* in F508del complex alleles in the context of corrector therapy and in combination with activators

Sara Bitam, Nathalie Serval, Natacha Martin, Pascale Fanen, Aleksander Edelman, Alexandre Hinzpeter

P18 A LC-MS lipidomics study in lung lavage samples from cf infants reveals differences between severe and mild lung, identifying new therapeutic targets

Bob Scholte, Mieke Veltman, Slavik Koval, Rob Vreeken, Marta Stolarczyk, Amy Harms, Harm Tiddens, Hetty Janssens, Stephen Stick

P19 The epithelial transcription factor KLF4 affects the traffic of CFTR

Ines Pankonien, Sara Afonso, Margarida Quaresma, Shehrazade Dahimène, Hugo Botelho, Ana Cachaço, Karl Kunzelmann, Margarida Amaral

P20 Defective PPAR γ signaling and increased mucin/bicarbonate expression in the regulation of human airway epithelial cell repair

Joanna Bou Saab, Ines Boal-Carvalho, Marc Bacchetta, Marc Chanson

P21 Orphan G-protein coupled receptor CELSR3 is an epithelial marker downregulated in cystic fibrosis

Arnaud Dykmans, Wendy Delbart, Barbara Dhooghe, Teresinha Leal, Sabrina Noel

P23 Role of miRNAs in the CF pathology

Jennifer Bonini, Jessica Varilh, Raphael Chiron, Emmanuelle Brochiero, Mireille Claustres, Magali Taulan-Cadars

P24 MiR-210 and miR-155 are upregulated in cystic fibrosis cells and involved in Fe-S protein assembly via ISCU downregulation as well as hemeoxygenase-1 expression via BACH1

Shashi Chillappagari, Virajith Garapati, Poornima Mahavadi, Oliver Stehling, Roland Lill, Bernd Schmeck, Markus Henke

P25 Differential gene activation in normal and cystic fibrosis human bronchial epithelial cells upon *Pseudomonas aeruginosa* infection

Viviane Balloy, Soumaya Boudaya, Jean Yves Coppee, Marie Agnès Dillies, Hugo Varet, Caroline Proux, Harriet Corvol, Michel Chignard, Loic Guillot

P26 *Pseudomonas aeruginosa* adaptation to cystic fibrosis airways shapes the host response in mice during the progression of airway disease

Camilla Riva, Cristina Cigana, Nicola Ivan Loré, Ida De Fino, Lorenza Spaguolo, Barbara

Sipione, Giacomo Rossi, Alessandra Bragonzi

P27 Dissection of host susceptibility to *Pseudomonas aeruginosa* lung infection in mice

Maura De Simone, Lorenza Spagnuolo, Nicola Ivan Lorè, Cristina Cigana, Ida De Fino, Karl Broman, Fuad A. Iraqi, Alessandra Bragonzi

P28 Identification of metalloprotease activity in *P. aeruginosa* clinical strains and its sensitivity to azithromycin treatment through a metalloprotease activity assay based on flow cytometry

Gabriella Bergamini, Angela Sandri, Baroukh Assael, Paola Melotti, Claudio Sorio

P29 The role of mucus and mucin in mediating *Pseudomonas aeruginosa* infection and virulence in cystic fibrosis

Patrick Moore, Paul McNally, Valerie Urbach, Marguerite Clyne

P30 Azithromycin effect on lung inflammation induced by *Pseudomonas aeruginosa* released proteases as shown by in vivo imaging in IL-8 transiently transgenized mice

Gabriella Bergamini, Fabio Stellari, Angela Sandri, Francesca Ruscitti, Gino Villetti, Claudio Sorio, Baroukh Assael, Paola Melotti, Maria Lleo

P31 *Pseudomonas aeruginosa* elastase LasB modulates CFTR expression, innate immune responses and epithelial repair in airway epithelial cells

Vinciane Saint-Criq, Fabien Bastaert, Aurélie Hatton, Aleksander Edelman, Jean-Michel Sallenave

P32 The role of lipoxin A₄ on tight junctions and antimicrobial peptide production by CF airway epithelial cells

Gerard Higgins, Valerie Urbach, Paul McNally, Juliette Simonin

P33 Lipoxin A₄ regulates cAMP dependent ion transport and cell migration in the colonic cell line T84

Monika Hollenhorst, Valerie Urbach

P34 Airway epithelial cell integrity protects from cytotoxicity of *P. aeruginosa* quorum-sensing signals

Davide Losa, Thilo Köhler, Marc Bacchetta, Joanna Bou Saab, Maud Frieden, Christian van Delden, Marc Chanson

P35 A new synthetic compound inhibits the expression of Quorum-Sensing regulated genes and biofilm formation in *Pseudomonas aeruginosa*

Aurelie Furiga Chusseau, Barbora Lajoie, Salome El Hage, Genevieve Baziard, Christine Roques

P36 An investigation of the role of *Burkholderia cenocepacia* lipopolysaccharide (LPS) O-antigen in modulating the host pro-inflammatory response

Luke McGuigan, Siobhán McClean, Máire Callaghan

P38 Elucidation of the role of peptidoglycan-associated lipoprotein (BCAL3204) in

the virulence of *Burkholderia cepacia* complex

Ruth Dennehy, Cristobal Mujica, Maria Romano, Rita Berisio, Miguel Valvano, Máire Callaghan, Siobhán McClean

P39 The host response to iron sequestration by *B. cenocepacia*

Niamh Whelan, Nicola Maloney, Mark Thomas, Sean Doyle, Máire Callaghan

P40 Neutrophil elastase-cleaved SPLUNC1 disrupts *Burkholderia cepacia* biofilms better than intact SPLUNC1

Jean Tyrrell, Saira Ahmad, William Walton, Matthew Redinbo, Robert Tarran

P41 Development of new inhibitors of the non-lysosomal β -glucosylceramidase GBA2 as possible anti-inflammatory agents for CF lung disease

Massimo Aureli, Silvia Munari, Susanna Khalil, Nicoletta Loberto, Valentina Lovato, Valentino Bezzerri, Anna Tamanini, Rosaria Bassi, Domitilla Schiumarini, Maria Grazia Giri, Caroline Norez, Frederic Becq, Boris Vauzeilles, Sandro Sonnino, Giulio Cabrini, Maria Cristina Dehecchi

P42 Passage-dependent effects of CF bronchial epithelial cells

Hollie Wilson, Madeleine Ennis, Bettina Schock

P43 Investigating the supramolecular organisation of respiratory mucins

Gareth Hughes, Richard Collins, Robert Ford, David Thornton

P44 The P2X₄ receptor is upregulated in human trachea cells with chronic IL-13 treatment and influences mucin secretion

Kristin Thompson, Kathrin Neuland, Achim Riecker, Melanie Timmler, Hannah Schmidt, Oliver Wittekindt, Paul Dietl, Manfred Frick

P45 Imbalance of mucus homeostasis in chronic pancreatitis

Anita Balázs, Julia Duerr, Zhe Zhou-Schuckow, Jolanthe Schatterny, László Tiszlavicz, István Németh, Balázs Kui, Zoltán Rakonczay, Marcus Mall, Péter Hegyi

P46 Structure activity relationship for OligoG-induced normalization of the CF mucus phenotype

Anna Ermund, Lauren Meiss, Christian Recktenwald, Edvar Onsøyen, Philip Rye, Arne Dessen, Yngvar Berg d.y., Astrid Hilde Myrset, Gunnar Hansson

P47 Mechanism-based screens lead to the identification of complementary pharmacological correctors of F508del CFTR

Feng Liang, Haibo Shang, Nikole Jordan, Eric Wong, Veit Guido, Gergely Lukacs, Jerome Mahiou, Hermann Bihler, Martin Mense

P48 Predicting the individual clinical efficacy of CFTR-modulating drugs using rectal cystic fibrosis organoids

J F Dekkers, Gitte Berkers, E Kruisselbrink, A M Vonk, H R de Jonge, S Heida-Michel, M Geerdink, H M Janssens, I Bronsveld, E E S Nieuwenhuis, R H J Houwen, F P Vleggaar, J C Escher, Y B de Rijke, K M de Winter-de Groot, H Clevers, C K van der Ent, J M Beekman

P49 Measurements of functional responses in human primary bronchial and nasal cells as a basis for personalized therapy for cystic fibrosis

Nikhil Awatade, Inna Uliyakina, Maria Margarida Ramos, Margarida Amaral

P50 Rescuing F508del-CFTR in airway epithelial cells using a novel traffic-based high content microscopy screening platform

Hugo Botelho, Shehrazade Dahimène, Inna Uliyakina, Beate Neumann, Christian Tischer, Rainer Pepperkok, Margarida Amaral

P51 Anchoring to the actin-cytoskeleton is not essential to retain functional F508del-CFTR at the plasma membrane

Ana Matos, Joana Pereira, Inna Uliyakina, Margarida Amaral, Paulo Matos

P52 Cholapods mediate anion transport across epithelial cell membranes

Hongyu Li, Germinal Magro, Luke Judd, Peter Brotherhood, David Sheppard, Anthony Davis

P53 Identification of novel potentiator compounds that do not decrease membrane density of Δ F508-CFTR

Emily Langron, Paola Vergani

P54 Identification of CF mutations in non-coding regions: design of antisense oligonucleotides for a targeted therapeutic approach

Jessica Varilh, Jennifer Bonini, Caroline Raynal, Corinne Thèze, Emmanuelle Beyne, Jean-Pierre Altieri, Fanny Verneau, Marie-Pierre Audrezet, Claude Férec, Thierry Bienvenu, Emmanuelle Girodon, Sylvie Tuffery-Giraud, Marie Des Georges, Mireille Claustres, Magali Taulan-Cadars

P55 Characterization and correction of CFTR mutations bearing premature termination codons (PTCs)

João Amorim, Verónica Felício, Susana Igreja, Anabela Ramalho, Margarida Amaral

P56 *In vitro* correction of the CFTR 2657+5G>A splicing mutation by an antisense oligonucleotide

Susana Igreja, Margarida Amaral

P57 CRISPR/Cas-9 NHEJ based knock-out of CFTR class I mutations to restore splicing

David Sanz, Jennifer Hollywood, Kendra Tosoni, Stephen Land, Anil Mehta, Patrick Harrison, Diane Cassidy

P58 Modulating rescue of Δ F508 CFTR in human airway epithelial cultures

Imron Chaudhry, Deborah Cholon, Susan Boyles, Nancy Quinney, Pheris Karanja, Silvia Kreda, Jennifer Guimbellot, Martina Gentsch

P59 Trafficking in CFTR therapies

Sahar Al-Ayyoubi, Moises Chavez, Jeanine Amacher, Kristi Cihil, Agnes Swiatecka-Urban, Roxanna Barnaby, Bruce Stanton, Prisca Boisguerin, Rudolf Volkmer, Nico Derichs, Dean Madden

P60 Interactions of cytokeratin 8 with misfolded Phe508del-CFTR and Z-alpha-1 antitrypsin – role in protein trafficking and/or degradation

Iwona Pranke, Sara Bitam, Kamil Tomaszewski, Grazyna Faure, Imran Haq, Harald Herrmann, David Lomas, Isabelle Sermet-Gaudelus, Aleksander Edelman

P61 preclinical development of a novel corrector for cystic fibrosis

Melanie Faria da Cunha, Aurelie Hatton, Vincent Jullien, Aleksander Edelman, Isabelle Sermet-Gaudelus

P62 Rescue of N1303K chloride currents by small molecule correctors and transcomplementation

Daniele Rapino, Inna Sabirzhanova, Miquéias Lopes-Pacheco, Rahul Grover, William Guggino, Liudmila Cebotaru

P63 Mechanism of action of rattlesnake phospholipase A₂ on CFTR dysfunction

Grazyna Faure, Naziha Bakouh, Stéphane Lourdel, Norbert Odolczyk, Aiswaraya Premchandrar, Nathalie Serval, Aurélie Hatton, Maciej Ostrowski, Haijin Xu, Frederick Saul, Christelle Moquereau, Iwona Pranke, Gabrielle Planelles, Jacques Teulon, Piotr Zielenkiewicz, Michal Dadlez, Gergely Lukacs, Isabelle Sermet-Gaudelus, Mario Ollero, Pierre-Jean Corringier, Aleksander Edelman

P64 Disruption of a PI3K γ /PKA/PDE signaling complex augments cAMP/PKA-signaling and CFTR activity in non-CF and Δ F508-CF airway epithelial cells

Wito Richter, Marco Conti, Emilio Hirsch, Alessandra Ghigo

P65 Enhanced CFTR modulation with S-nitrosoglutathione reductase inhibitor in combination with CFTR corrector and potentiator

Peter Bove, Kirsten Look, Sherif Gabriel

P66 Analysis of the chronic effects of lumacaftor and ivacaftor on F508del-CFTR single-channel gating and stability

Yiting Wang, Zhiwei Cai, David Sheppard

P67 Studies of the structural components of VX-770 necessary for its activity

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Robert Rauscher, Sebastian Kirchner, Zoya Ignatova

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Gabriella Bergamini, Denise Peserico, Teresinha Leal, Marco Tagliasacchi, Elena Sorio, Stefano Ceri, Claudio Sorio, Baroukh Assael, Jeffrey Wine, Paola Melotti

P77 Constitutive activation of the Ca²⁺-activated chloride channel TMEM16A

Paolo Scudieri, Arianna Venturini, Oscar Moran, Luis Galiotta

P78 TMEM16A is required for intestinal Cl⁻ secretion and activation of CFTR

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Johanna Salomon, Stephan Spahn, Xiaohui Wang, Joachim Füllekrug, Carol Bertrand, Marcus Mall

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Barbara Dhooghe, Sabrina Noël, François Huaux, Nadtha Panin, Teresinha Leal

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Sara Cuevas Ocaña, Magomet Aushev, Michael Gray, Julia Reichelt

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25 March — 18:00–19:00

Opening Keynote Lecture

Protective HCO_3^-

Paul M. Quinton

Dept. Pediatrics, UC San Diego School of Medicine, and Biomedical Sciences, UC Riverside School of Medicine, and Rady Children's Hospital

Since Sydney Farber used the term 'mucoviscidosis' in 1945, the medical scientific community has attributed the morbidity and mortality of what we now commonly call Cystic Fibrosis (CF) to pathogenic mucus, especially in the lungs. And since then we continue to be ever more challenged by evolving knowledge that describes CF. From his recognition that CF is a multi-organ disease characterized by purulent as well as mucoid plugs in secretory organs to the later recognized basic, but seemingly unrelated, abnormalities in fluid and electrolyte transport and then to a cloned gene for an anion channel (CFTR), we presently arrive at the intersection of mucus and failed HCO_3^- secretion to return to mucoviscidosis and pathogenesis in CF. In this presentation, we will try to briefly review some of the events that have shaped our thinking as to what CF is and also try to integrate the secretion of mucin, fluid, and HCO_3^- in the tissue most critical to health in CF patients; i.e., the small airways.

In contrast to larger airways, small airways have few, or no, glands to supply fluid secretion, nor cartilaginous rings to support patency, nor high velocity air flow with cough for protective clearance of airway surfaces. We will present evidence that the small airways meet their challenge to remain clear and open with an epithelial lining that is comprised of discrete regions that secrete fluid within local pleats that is then reabsorbed in adjacent folds. Moreover, like other target tissues in CF, we find that the small airways secrete HCO_3^- as well as mucus and that HCO_3^- is required for normal mucus maturation and transport. The anion is also critical to other modes of innate defense. We will consider the special arrangement of pleats and folds in these epithelial tubes in the context of supporting normal airway clearance and innate defense at this vulnerable level of the bronchial tree. Thus, we find that without CFTR dependent HCO_3^- secretion, mucoviscidosis occurs in the small airways of CF lungs and, in general, in other pathologically affected or destroyed tissues.

In short, we will examine a new paradigm for integrating the structures and functions of small airways to better understand airway surface clearance in this critical target of CF disease. Such understanding may support new strategies for managing and treating Cystic Fibrosis and possibly other lung diseases.

26 March — 08:45–10:30

Symposium 1 - CFTR folding and structure

S1.1 CFTR purification and advances towards a high resolution structure.

Robert C. Ford, Xin Meng, Naomi Pollock, Natasha Cant, Vasileios Kargas

University of Manchester, Manchester, United Kingdom

We have expressed the full-length CFTR protein in yeast cells (*S. cerevisiae*). Versions of the protein incorporating the two most common mutations (F508del and G551D) have also been expressed as well as wild-type CFTR. Human F508del CFTR expresses surprisingly well in yeast, perhaps because of the low growth temperature (20°C). Once expressed, it can be purified with similar yields and purity to the wild-type protein. The G551D version of the protein appears to be the most stable and homogeneous construct after purification, as judged by analytical size-exclusion chromatography and thermal denaturation experiments employing a Cys-reactive dye. However the G551D mutation inactivates the protein in terms of its ATPase activity (and channel activity). This lack of activity can be rescued in patients by the potentiator ivacaftor (VX-770). The F508del version of the protein appears to be the least stable of the constructs, although this defect can be partially rescued by the corrector compound lumacaftor (VX-809). The properties of full-length CFTR also depend on the detergent employed during its purification. The commonly employed non-ionic detergent dodecyl maltoside (DDM) allows retention of ATPase activity ($k_{cat}=0.25s^{-1}$), but for structural studies, the material is compromised in terms of its homogeneity, as confirmed by negative-stain electron microscopy. In contrast the anionic lyso-lipid detergent lyso-phosphatidyl glycerol (LPG-14) allows purification and good homogeneity, even to the point of allowing the formation of small clusters with crystallinity. However this detergent results in loss of ATPase activity (10-20% of that in DDM). I will describe progress towards obtaining CFTR with the ideal compromise between homogeneity and activity that will be used in 3D crystallization trials. Electron microscopy studies of purified WT, F508del and G551D CFTR have been carried out in both LPG and DDM detergents and some insights into the structure of CFTR have been derived as a result. These have been used to help interpret data derived from small-angle X-ray scattering (SAXS) studies and vice-versa (in collaboration with Dr Oscar Moran, Istituto di Biofisica, CNR, Genova, Italy). In the absence of a high resolution structure of CFTR I will examine the correspondence between the current highest detail structural map of CFTR (at about 9 Angstrom resolution) versus the best homology models available for the protein. Likewise, I will examine new high resolution structures for potential CFTR homologs and whether they can give new insights into its structure and function. The prospects and limitations for in-silico structures of CFTR will be discussed.

Supported by the Cystic Fibrosis Foundation (USA), the bbsrc (UK), China Scholarship Council (CSC) and ASTAR (Singapore/Manchester) graduate program.m.

S1.2 Towards an understanding of NBD1 and NBD2 heterodimerization

Andrew Chong^{1,2}, Robert Vernon^{1,2}, Rhea Hudson^{1,2}, Jennifer Dawson^{1,2}, Alaji Bah^{1,2}, Julie Forman-Kay^{1,2}

¹University of Toronto, Department of Biochemistry, ²Program in Molecular Structure and Function, Hospital for Sick Children

Nucleotide binding by the first and second nucleotide binding domains (NBD1 and NBD2), heterodimerization of NBD1 and NBD2 and hydrolysis of the nucleotide at the heterodimer interface is thought to regulate opening and closing of the pore formed by the membrane spanning domains (MSDs) of CFTR. Based on CFTR NBD1 and other ABC transporter NBD crystal structures, the NBDs are presumed to heterodimerize in a "head to toe" manner with two nucleotide molecules sandwiched at the interface. The intrinsically disordered regulatory segments, including the regulatory insertion of NBD1 (RI) and R region, have been postulated to inhibit NBD heterodimerization, with inhibition being relieved by phosphorylation of these regions. The disordered C-terminus of CFTR may also participate in NBD heterodimerization. Neither biochemical nor structural studies have been successful in observing a direct interaction between the isolated NBD1 and NBD2 proteins, hindering our structural understanding of heterodimerization and the gating cycle. This is due in part to the extremely poor behavior of isolated NBD2. We use computational and biophysical tools to address NBD heterodimerization with the goal of understanding how this heterodimerization is controlled. Bioinformatic and structure simulation tools were used to suggest mutations that would improve the behavior of NBD2. As a result, we are now able to produce NBD2 with significantly improved behavior for biophysical characterization including fluorescence and NMR studies. We have previously presented data from NMR studies that provide evidence of NBD1 homodimerization, which we found to be impaired by the F508del mutation. However, preliminary NMR studies failed to provide concrete evidence of NBD1:NBD2 heterodimerization. To address this, we are using paramagnetic relaxation enhancement (PRE) to further probe heterodimerization. Soluble single cysteine mutants of NBD1 were generated to allow attachment of a single cysteine-linked spin label. Control experiments verified that these PRE experiments enable observation of NBD1 homodimers. We provide a progress update on PRE and fluorescence experiments designed to observe NBD heterodimerization. With these new reagents, we aim to probe the effects of nucleotide binding, F508del and other disease causing mutations, the C-terminus, the RI and the R region (both phosphorylated and non-phosphorylated) on NBD1:NBD2 dimerization. This work will also enable studies of small molecule modulators targeted at the NBD dimer interface. This work was funded by grants from the Cystic Fibrosis Foundation Therapeutics and Cystic Fibrosis Canada.

S1.3 ABCs in Adaptive Immunity and Viral Immune Evasion

Robert Tampé

Goethe University Frankfurt, Germany

The recognition and elimination of virally or malignantly transformed cells is the pivotal task of the adaptive immune system. For immune surveillance, a snapshot of the cellular proteome is displayed on major histocompatibility (MHC) class I molecules as immunodominant epitopes for recognition by cytotoxic CD8⁺ T-lymphocytes. The knowledge about the track from the equivocal protein to the presentation of peptides has greatly expanded, leading to an astonishingly elaborated understanding of the MHC I peptide loading pathway. This seminar will report on this complex process, which rests on ABC transporters, chaperones, and ER quality control. The contribution of the individual proteins and subcomplexes as well as the architecture and dynamics of the peptide-loading complex will be discussed, including mechanisms of viral immune evasion.

P4

S1.4 Motions of CFTR membrane spanning helices related to channel gating

Jhuma Das, Andrey Aleksandrov, Liying Cui, Lihua He, John Riordan, Nikolay Dokholyan

University of North Carolina at Chapel Hill, Biochemistry & Biophysics, Chapel Hill, United States

The paucity of CFTR high-resolution 3D structural information has impeded elucidation of its mechanism of action and understanding of the impact of disease causing mutations. Homology models developed employing ABC exporters of known structure as templates have contributed somewhat towards realization of these objectives. However because of the very low sequence similarity between CFTR and the templates it is not expected that they very precisely mirror each other, especially in the membrane domains. Nevertheless, data from the influence of mutagenesis, labeling, and cross-linking experiments (Linsdell P. *Mol Membr Biol.* 2014, 31:1-16.) suggest that the overall arrangements of membrane spanning helices (TMs) is consistent with that predicted by the models. However, there is little information about the motions of these helices that may be involved in channel gating. To address this issue we have iteratively applied computational modeling tools including discrete molecular dynamics simulations to reconstruct robust structural models of CFTR by introducing cysteine cross-linking/single channel measurements as experimental constraints. The data reveal that the crosslinking of a key residue in TM6 (R352C) with one in TM12 (W1145C) locks the channel closed. Consistent with the previous finding that R352 forms salt bridge with D993 in TM9 (Cui G. et al. *J Biol Chem.* 2013, 288:20758-67), our data implicates that these residues could be reversibly cross-linked. However, the functional impact of this pair could not be evaluated as the channel closed upon cross-linking. Strikingly, spontaneous oxidative disulfide linkage between cysteines replacing residues near the extracellular ends of TMs within the same “wing” of the modeled ABC structures and not expected to primarily influence OWF-IWF transitions reversibly locked the channel closed (e.g., D112C/T1122C and A326C/V905C) as did pairs across the two “wings” that might be expected to have such an influence (eg. L333C/G1127C). From these integrated computational and experimental analyses, we conclude that channel gating transitions depend on motions between multiple membrane helices and fixation in a stable open state by a single inter-helical restriction may not be readily achieved.

(Supported by the NIH and CFF)

P1

S1.5 Molecular dynamics simulations on the wild-type and F508del CFTR 3D models

Brice Hoffmann¹, Jean-Paul Mornon¹, Pierre Lehn², Isabelle Callebaut¹

¹IMPMC, Sorbonne Universités, Université Pierre et Marie Curie-Paris 06, CNRS UMR 7590, MNHN, IRD, Paris, France, ²Université de Bretagne Occidentale, INSERM U 1078, Brest, France

In the absence of experimental 3D structures at atomic resolution for the entire CFTR protein, we and others have built homology models using as templates the experimental 3D structures of ABC exporters. These models provided significant insights into the structural and functional characteristics of CFTR, and were enriched by several molecular dynamics (MD) simulations, which now offer a description of the possible architecture of the anion channel in both the open and closed forms. Moreover, our recent MD simulation performed on wild-type CFTR suggests that the region in the first nucleotide-binding domain (NBD1) where F508 resides undergoes significant flexibility that allows promoting the open conformation of the channel through its interaction with the fourth intracellular loop (ICL4) (1).

Here, we specifically analysed a model we made of CFTR carrying the F508 deletion. This deletion results in a temperature-sensitive folding defect that impairs protein maturation and chloride channel function (2). It is however known that second-site suppressor mutations partially reverse these effects (3). Especially at the level of the ICL4:NBD1 interface, the R1070W measurably promotes the trafficking of the F508del protein and partially rescues the F508del CFTR phenotype. In order to understand, at the molecular level, the mechanisms by which this mutation impacts intermolecular interactions, we thus carried out MD simulations on the CFTR F508del/R1070W double mutant. These suggest that the side chain of the R1070W promotes a local reorganization of the NBD1 region where the F508 deletion is located (AA 498 to 515), making it able to fold as observed in the wild-type protein (1), whereas the channel can achieve a full open conformation.

We also wish to study, through MD simulations, the effect of other second-site suppressor mutations as well as of the simultaneous genetic stabilization of NBD1 energetics and NBD1-ICL4 interface, leading to robust and synergistic rescue of CFTR F508del folding and function (4,5). These theoretical studies should provide insightful information on the molecular mechanisms necessary for the F508del protein to be able to fold and function properly, which could be used to design efficient correctors.

1. Mornon JP*, Hoffmann B*, Jonic S, Lehn P., Callebaut I. 2015 Cell Mol Life Sci in press.
2. Thibodeau PH et al. 2010 J Biol Chem 285: 35825-35835.
3. Liu X, O'Donnell N, Landstrom A, Skach WR, Dawson DC 2012 Biochemistry 51:5113-5124.
4. Rabeh WM et al. 2012 Cell 148: 150-163.
5. Mendoza et al. 2012 Cell 148: 164-174.

This work is supported by the French Association Vaincre La Mucoviscidose

P2

S1.6 Stability of CFTR and the effects of Cystic fibrosis-causing mutations in humans

Xin Meng, Xiaomeng Wang, Naomi Pollock, Tracy Rimington, Robert Ford

University of Manchester, Manchester, United Kingdom

F508deletion and G551D in the cystic fibrosis transmembrane conductance regulator (CFTR) protein are the most frequent cystic fibrosis (CF)-causing missense mutations. Drugs VX-809 (a corrector) and VX-770 (a potentiator) are being used in clinical trials to target these mutations. Human wild-type (WT), F508del and G551D full-length CFTR were expressed in *S. cerevisiae* and purified in detergents n-dodecyl β -D-maltopyranoside (DDM) or lyso-phosphatidyl glycerol (LPG). For the first time, the thermal stability of the mutated proteins versus WT CFTR was studied in the absence and presence of the two drugs and nucleotides. We show that F508del CFTR is considerably less stable than WT and G551D versions of the protein. This instability can be partly rescued by the VX809 drug. The data has considerable significance for current clinical trials and our understanding of the molecular defect in CF. We have developed a medium throughput assay to detect small molecules that can correct CFTR stability. This leads to a simple model for the effects of the mutations at physiological temperatures and possible routes for therapy via small molecule correctors. The screen has also allowed us to identify conditions in which CFTR is relatively stable. This information is being fed into crystallization trials aimed at the determination of the high resolution structure of the full-length protein. We have been able to produce the protein in significant quantities and at concentrations well above 10 mg/ml.

26 March — 11:00–12:45

Symposium 2 - Regulation of CFTR activity

S2.1 Revertant mutants modify, but do not rescue, the gating defect of the cystic fibrosis mutant G551D-CFTR

Zhe Xu¹, Luísa S. Pissarra², Carlos M. Farinha², Jia Liu¹, Zhiwei Cai¹, Patrick H. Thibodeau³, Margarida D. Amaral²,
David N. Sheppard¹

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Programme Note: Abstract details are not authorised for publication

S2.2 The CFTR channel as an allosteric protein: bypassing the normal requirements for ATP binding and R domain phosphorylation

Kevin Kirk

University of Alabama at Birmingham, USA

CFTR channel activity normally is tightly controlled by ATP binding to its NBDs and PKA phosphorylation of its R domain. However, it is possible to engineer mutations at multiple locations in the polypeptide that increase channel activity in the absence of ATP binding or R domain phosphorylation. This capability to produce gain of function (GOF) mutations supports the feasibility of developing allosteric drugs that activate CF mutant channels by bypassing the requirements for ATP binding or phosphorylation. Such GOF mutations also are useful tools with which to explore the links between channel gating, the ATP occupancies of the NBDs and R domain phosphorylation. I will discuss 3 classes of GOF mutations whose locations span the CFTR pore from the intracellular loops to the extracellular surface. These mutations locate to residues that are conserved among other ABCC transporters; notably, the MRP pumps. Mutations of each class exert GOF effects on CFTR gating in excised membrane patches; namely, increased activity in the absence of ATP, stronger activation by a normally weak agonist (AMP-PNP) and functional rescue of channels that are unresponsive to ATP (e.g., G551D). GOF mutations also reciprocally increase the sensitivity of CFTR activation to both ATP and PKA and rescue the ATP sensitivity of an NBD2 mutant that is defective for ATP binding (Y1219G). This reciprocity between channel gating, ATP sensitivity and R domain phosphorylation occurs even for mutations that are located far away from the NBDs and the R domain (e.g., the extracellular end of TM6). Such reciprocal effects of GOF mutations on ligand sensitivity are predicted by the types of allosteric schemes that explain the activation of neurotransmitter-gated channels or hormone receptors. Corresponding mutations at conserved locations in two yeast MRPs (Yor1p and Ycf1p) also rescue transport by analogous ATP binding mutants of these pumps presumably by a similar mechanism of reciprocity. Finally, by combining GOF mutations from different classes we can produce CFTR channels with high open probabilities ($P_o > 0.5$) in excised membrane patches in the absence of any ATP or exogenous PKA. These additive effects are consistent with each GOF mutant class having a distinct structural effect on the channel; an interpretation that is supported by molecular dynamics simulations. Our findings support the following conclusions: (i) CFTR activation by ATP binding and R domain phosphorylation shares allosteric principles with conventional ligand-gated channels and hormone receptors; (ii) the CFTR channel and MRP pumps use similar allosteric mechanisms to couple the conformations of their translocation pathways to the ATP occupancies of their NBDs and (iii) it is possible to engineer CFTR channels that are highly active in the absence of ATP and/or PKA, which reinforces the feasibility of discovering new allosteric drugs that bypass defects in ATP binding or phosphorylation.

S2.3 Structural dynamics of the CFTR pore opening conformational transition

Ben Sorum, Dávid Czégé, László Csanády

Semmelweis University, Budapest, Hungary

CFTR channel activity is regulated by phosphorylation of its intracellular regulatory (R) domain and ATP binding/hydrolysis at its two conserved cytosolic nucleotide binding domains (NBDs). Following phosphorylation by protein kinase A (PKA), in the presence of MgATP, gating of the CFTR channel pore is characterized by bursts of openings. CFTR bursting activity follows an irreversible cycle: initiation of a burst coincides with formation of an intramolecular NBD1/NBD2 heterodimer strongly held together by two ATP molecules occluded at the dimer interface, while termination of a burst coincides with disruption of the NBD dimer, normally following hydrolysis of one of the two bound nucleotides (*Nature* 433:876-880). The rate limiting step of the overall cycle is the slow pore opening step, characterized by a high-enthalpy transition state (*J Gen Physiol* 128:523-533), but the molecular details of this strained short-lived structure, and the relative timing of molecular motions that lead from the open-dimer/closed-pore to the tight-dimer/open-pore conformation, are unknown. We report here a thermodynamic study of the opening conformational transition using the rate-equilibrium free energy relationship (REFER) approach. Because REFER analysis provides no information on non-equilibrium systems (*J Gen Physiol* 134:129-136), we use a CFTR background construct in which mutation of the NBD2 Walker B aspartate prevents ATP hydrolysis, and thus reduces channel gating to a simple equilibrium process. Our results provide direct information on the relative timing of gating motions and on structural details of the opening transition state.

S2.4 Anoctamins control epithelial Cl⁻ secretion by CFTR

Inês Cabrita, Roberta Benedetto, Podchanart Wanitchakool, Karl Kunzelmann, Rainer Schreiber

University of Regensburg, Institute of Physiology, Regensburg, Germany

In cystic fibrosis, defective Cl⁻ secretion by mutant CFTR might be compensated by alternative chloride channels such as Ca²⁺ activated TMEM16 Cl⁻ channels. TMEM16A (anoctamin 1, ANO1) is expressed in epithelial tissues relevant to cystic fibrosis, like epithelial cells of airways, intestine and pancreas. We examined whether TMEM16A forms an independent secretory Cl⁻ channel or whether it operates in conjunction with CFTR, as previous work suggested. In mouse intestine lacking expression of TMEM16A, Ca²⁺ dependent Cl⁻ secretion was significantly reduced, although TMEM16A was found to be expressed in the basolateral membrane of enterocytes. These data indicated that i) CFTR might be the only apical exit pathway for mouse intestinal Cl⁻ secretion, similar to human intestine, ii) TMEM16A may facilitate Ca²⁺ signaling to activate basolateral Ca²⁺ dependent K⁺ channels to increase driving force for CFTR dependent Cl⁻ secretion, and iii) TMEM16A controlled Ca²⁺ signaling possibly activates apical CFTR by inhibition of Ca²⁺-dependent phosphatases and/or activation of PKC. We further examined in a recombinant cell system whether only TMEM16A or also other proteins of the TMEM16 family change intracellular Ca²⁺ signaling. Therefore we overexpressed ANO1, 4, 5, 6, 7, 8, 9 and 10 in HeLa cells and measured changes of intracellular calcium concentration close to the plasma membrane, using the plasma membrane targeted calcium sensitive GFP protein. We found that overexpression of ANO 1, 5, 6 and 10 enhanced intracellular Ca²⁺ signaling elicited by purinergic stimulation with 100 μM ATP. We further studied cellular localization of overexpressed ANO10 in HEK293 cells. Membrane biotinylation and costaining with ER tracker indicated that most of ANO10 was located in the endoplasmic reticulum (ER) rather in the plasma membrane. However, cellular localization was depending on cellular proliferation. We propose that ANO10 and possibly other anoctamins can act as a counter ion channel for calcium store release from the ER, if partially located in the ER. Taken together, anoctamins may control epithelial Cl⁻ transport either by acting as apical Cl⁻ exit channels or by facilitating CFTR dependent Cl⁻ secretion due to enhanced intracellular Ca²⁺ signaling.

Supported by DFG SFB699, Cystic Fibrosis Trust grant SRC003.

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S2.5 Role of miRNAs in the CF pathology

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Our group is interested in the identification of regulatory elements, including transcription factors and miRNAs, that control the *CFTR* mRNA level.

We recently characterized miRNAs acting only in lung adult cells compared to data from primary fetal cells. Our finding allowed us to envision new therapeutic tools focused on miRNAs. We used an experimental model based on scratching of nasal epithelium from healthy individuals or patients with cystic fibrosis and homozygous for the p.Phe508del *CFTR* mutation, cultured in an air-liquid interface (ALI). This model allows testing molecules for the correction of *CFTR*-mutation consequences. We demonstrated that introduction of oligonucleotides (which specifically block the binding of miRNAs) increased the *CFTR* mRNA and *CFTR* protein levels on CF nasal epithelium and enhanced *CFTR* function in CFBE14o- cells*.

It is becoming increasingly clear that in addition to their role in the *CFTR* mRNA expression, miRNAs play a role in manifestations of CF lung disease. For instance, miR-101 and miR-145 are deregulated in vivo in CF bronchial epithelium.

Material and methods: To identify other putative targets, we assessed the gene expression pattern of miRNAs in ALI epithelium cultured from CF patients (p.Phe508del homozygous) or healthy. We compared two approaches i.e. miRNA sequencing (miRseq by using MiSeq, Illumina) and TLDA system (TaqMan Low Density Array) and three models i.e. ALI epithelium from nasal, polyps or bronchial biopsies (n=4 per condition). For miRseq, three different programs were applied including MiSeqReporter, MiRanalyzer and sRNAbench.

Results: First results confirmed the high expression of the miR-449 family in ALI models previously described as key regulators ciliogenesis. In addition, these miRNAs are over-expressed in CF versus non-CF in two models. We also observed the deregulation of miR-101 and miR-27b in CF polyps and CF bronchial ALI. Treatment of ALI epithelium with inhibitors for some miRNAs is being tested. Role and targets of some deregulated miRNAs are also in progress.

Conclusion: Finding new regulatory players involved in CF physiopathology and/or controlling the *CFTR* mRNA level help us to envision new tools for Cystic Fibrosis therapy.

This work is supported by AFM-Téléthon and the association Vaincre la Mucoviscidose

- Transcription factors and miRNAs that regulate fetal to adult *CFTR* expression change are new targets for cystic fibrosis, Eur Respir J.2015 Jan; 45(1):116-28

P6

S2.6 EPAC1 activation by high levels of cAMP stabilizes CFTR at the membrane through interaction with NHERF-1

Miguel Lobo¹, João Fernandes¹, Margarida Amaral¹, Manuela Zaccolo², Carlos Farinha¹

¹Faculty of Sciences, University of Lisbon, BiolSI – Biosciences and Integrative Systems Institute, Lisboa, Portugal, ² University of Oxford, Department of Physiology, Anatomy & Genetics, Oxford, United Kingdom

Background: CFTR-mediated ion transport is stimulated by increase in subcortical cAMP levels through protein kinase A (PKA) activation thus triggering CFTR phosphorylation and channel opening. However, besides PKA, exchange proteins (EPACs) are also directly activated by cAMP by direct binding of this second messenger. EPACs are guanine nucleotide exchange factors (GEFs) for Rap family small GTPases, regulating cell-to-cell and cell-to-matrix adhesion, cytoskeleton rearrangements and cell polarization, processes which are dysregulated in CF. In response to cAMP, EPACs shift to the plasma membrane (PM) anchoring through ezrin-radixin-moesin (ERM) proteins, known to link CFTR to the actin cytoskeleton and to facilitate cAMP-driven CFTR activation by tethering PKA in their close proximity. This suggests that activation of both CFTR and EPACs might be spatially and temporally coincident, but little is known of the involvement of EPACs in CFTR biology.

Aim: We aimed to characterize the interaction between EPAC1 and CFTR and to elucidate the impact of EPAC1 on CFTR biogenesis, processing, trafficking and PM anchoring.

Methods: Western blot (WB), co-immunoprecipitation (co-IP), cell surface biotinylation, live cell imaging and FRET. Lung epithelial cell lines: A549 cells expressing wt- or F508del-mCherry-CFTR under a Tet-ON promoter, CFBEs stably expressing wt- or F508del-CFTR, Calu3 and HEK293T cells.

Results: Our results show that:

- (i) EPAC1 activation with the cAMP analogue 007-AM (a membrane-permeable EPAC-specific agonist not activating PKA) leads to its translocation from cytosolic and perinuclear regions to the PM, in CFBE cells.
- (ii) EPAC1 interacts with NHERF1 and this interaction is further promoted by EPAC activation.
- (iii) EPAC1 co-localizes and is present in protein complexes involving wt- or F508del-CFTR and this interaction is mediated by NHERF1 but not ezrin.
- (iv) 007-AM-activated EPAC leads to its increased association with CFTR and increased CFTR PM levels, without affecting CFTR steady-state processing efficiency (band C/band B ratio).
- (v) Increase in endogenous cAMP levels, through forskolin-activated adenylate cyclase (AC), increases the membrane levels of CFTR.
- (vi) EPAC1 knockdown (by siRNA or shRNA) leads to decreased of CFTR PM levels in different cell lines.
- (vii) Treatment with 007-AM promotes cell adhesion when compared to the DMSO control in CFBE cells expressing wt-CFTR, but not in CFBE cells expressing F508del-CFTR.

Conclusion: Our results establish that EPAC1 associates to CFTR in membrane complexes with NHERF-1, suggesting an involvement of EPAC1 in the late stages of CFTR biogenesis, and supporting a role for EPAC1 in enhancing CFTR traffic to the PM, making it a novel potential therapeutic target for CF. These data indicate that cAMP signalling regulates CFTR through two distinct but complementary pathways – PKA and EPAC1.

Work supported by FCT (Portugal) through MCTES EXPL/BIM-MEC/1451/2013 grant and PEst-OE/BIA/UI4046/2011 BioFig centre grant and by 2012 ERS Romain Pauwels Research Award. Authors thank CFF-USA for anti-CFTR antibody.

26 March — 16:00–17:45

Symposium 3 - CFTR and bicarbonate in CF disease

S3.1 Involvement of CFTR in glucose-induced electrical activities and insulin secretion in pancreatic islet β -cells

Jing Hui Guo, Ye Chun Ruan

The Chinese University of Hong Kong

Up to 50% of adult patients with cystic fibrosis (CF), a disease caused by mutations in the gene encoding the CF transmembrane conductance regulator (CFTR), develop CF-related diabetes (CFRD) with most patients exhibiting insulin insufficiency. However, the underlying cause remains poorly understood although destruction of the insulin-secreting pancreatic islets secondary to the obstruction of the pancreatic duct due to defective CFTR has long been considered the underlying cause. Defective exocrine pancreatic ductal HCO_3^- secretion is one of the hallmarks in CF and we have attempted to test whether defective exocrine pancreatic HCO_3^- secretion may lead to defective endocrine islet insulin secretion. However, pancreatic islet insulin secretion does not seem to respond significantly to the varying concentrations of extracellular HCO_3^- . The expression of CFTR in the islet β -cells prompted us to examine the role of CFTR in the regulation of glucose-dependent electrical activities and insulin secretion in β -cells. We have demonstrated that glucose-elicited whole-cell currents, membrane depolarization, electrical bursts or action potentials, Ca^{2+} oscillations and insulin secretion are abolished or reduced by inhibitors or knockdown of CFTR in primary mouse β -cells or RINm5F β -cell line, or significantly attenuated in CFTR mutant (DF508) mice compared to wildtype mice. VX-809, a corrector of DF508 mutation, successfully rescues the defects in DF508 β -cells. Interestingly, CFTR expression can also be upregulated by glucose. Thus, it appears that CFTR may respond to blood glucose acutely and transcriptionally and regulate glucose-induced electrical activities and insulin secretion in β -cells, defect of which may lead to CFRD.

S3.2 CFTR inhibition and elevated mucus concentration are key events in pancreatitis

Peter Hegyi

University of Szeged, Hungary

BACKGROUND

We have recently shown that disrupted level and function of cystic fibrosis transmembrane conductance regulator (CFTR) chloride channel plays a crucial role in the early phase of acute pancreatitis and apparent in chronic pancreatitis as well (Gastroenterology 2011¹, Gastroenterology 2015²). As we know that one of the leading histological features of chronic and recurrent pancreatitis is the formation of a protein gel in the fine pancreatic ducts³ and the main pathological feature of CF is the accumulation of thick and sticky mucus, it is almost needless to say that understanding the mucus homeostasis of the pancreas is highly important both in physiological and pathophysiological aspects.

HYPOTHESIS

Our hypothesis is that pancreatitis causing toxic factors, such as alcohol, tobacco and bile acids in addition to having a deleterious effect on ductal electrolyte transport also alter the regulation of mucus secretion. The combined effect will elevate the viscosity of pancreatic juice, create a pathological epithelial mucus phenotype leading to ductal cell damage, acinar cell atrophy and progressive tissue damage. In our current study we wish to prove our hypothesis that the imbalance of mucus homeostasis is a key and early event of the pathogenesis of pancreatitis.

RESULTS

We have analyzed expression of different mucin subtypes in mouse pancreata (*muc1*, *muc2*, *muc4*, *muc5ac*, *muc5b*, *muc6*) in collaboration with Marcus Mall (Heidelberg, Germany) and found that *muc6* is significantly upregulated in a chronic pancreatitis mouse model (induced by ~1000-fold) indicating that *muc6* protein is the main secretory constituent of mucus. Experiments on human pancreata revealed that mucus content is also significantly higher in patients suffering in chronic pancreatitis. Moreover, we investigated the tissue RNA level of *muc6* in normal and chronically inflamed pancreas. We have found that secretory mucoprotein *MUC6* is 17-fold overexpressed in human CP tissue.

FUTURE PERSPECTIVES

We would like to understand the time-course relationship between the major phenotype changes namely the secretory damage, inhibition of CFTR, the enhanced mucus production and fibrosis. This has crucial importance since these experiments will provide direct evidence that which is the earliest pathophysiological changes, moreover, it will reveal whether there is any cause-effect relationship between them or they take place parallel to each other.

REFERENCES

1. Pallagi, P. *et al. Gastroenterology* **141**, 2228--2239.e6 (2011).
2. Maléth, J. *et al. Gastroenterology* (2015). doi:10.1053/j.gastro.2014.11.002
3. Sarles, H. *et al. Observations on 205 confirmed cases of acute pancreatitis, recurring pancreatitis, and chronic pancreatitis. Gut* **6**, 545--559 (1965).

S3.3 CFTR and bicarbonate in CF disease

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The pancreas is among the most sensitive organs to the effects of CFTR dysfunction. CFTR is expressed in the pancreatic duct cells, and plays a critical role in sodium bicarbonate secretion. This fluid secretion flushes zymogens, the inactive form of pancreatic digestive enzymes, out of the pancreas and into the intestine. CFTR dysfunction leads to recurrent attacks of acute pancreatitis, and finally chronic pancreatitis linked to failed flushing of zymogens, intrapancreatic activation of trypsin and other digestive enzymes causing tissue autodigestion, severe inflammation and scarring. Stimulation of pancreatic exocrine secretion results in a brisk increase in the bicarbonate concentration of pancreatic juice. The mechanism of bicarbonate secretion by pancreatic duct cells has been debated for decades. The leading theory proposed that pancreatic duct cell CFTR facilitated chloride secretion across the apical (luminal) membrane to provide luminal chloride for bicarbonate secretion via a chloride-bicarbonate antiporter. We questioned this model, and proposed an alternate model with bicarbonate being secreted through CFTR with chloride conductance being limited by the absence of a chloride pathway on the basolateral membrane. Equation-based modeling suggested brisk bicarbonate secretion after CFTR activation, with luminal bicarbonate reaching high concentrations, as seen in humans. Functional impairment of pancreatic fluid secretion was predicted with selective dysfunction of bicarbonate conductance, but not chloride conductance. To test this hypothesis we recruited ~1000 subjects with chronic pancreatitis but without lung disease through the North American Pancreatitis Study II (NAPS2). We identified 9 CFTR variants, thought to be benign, that were in excess in the pancreatitis group. Functional studies demonstrated selective impairment in bicarbonate, but not chloride permeability and conductance after WNK1-SPAK activation. We conducted molecular dynamic modeling to understand the mechanism of these pathogenic CFTR variants, revealing 4 different mechanisms. A review of the NAPS2 case report forms phenotypes indicated that the bicarbonate-defective CFTR variants were associated with chronic rhinosinusitis and male infertility in pancreatitis patients, while these conditions were not increased in other pancreatitis patients. These data suggest a secondary syndrome within CF-associated diseases affecting the subset of organs that use CFTR for bicarbonate conductance rather than for secreting chloride.

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S3.4 Orphan G-protein coupled receptor CELSR3 is an epithelial marker downregulated in cystic fibrosis

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Université Catholique de Louvain, Louvain Centre for Toxicology and Applied Pharmacology, Brussels, Belgium

Airway epithelial barrier function is impaired in cystic fibrosis and this phenomenon has been linked to defective trafficking of F508del-CFTR. In addition, abnormal expression and localization of CFTR, even wild-type protein, may also be observed during epithelial remodeling and dedifferentiation. In both situations, the role of apico-basal polarity is crucial. Epithelial cells also exhibit Planar Cell Polarity (PCP) which has been described in mammal lung epithelial cells as a crucial mechanism controlling coordination, development and function of epithelial specialized structure in the plane of the epithelium. However, little is known about PCP in bronchial epithelial cells.

We demonstrated that human bronchial epithelial cells expressed several PCP core and effector proteins. In particular, we demonstrated that atypical cadherin CELSR3 (Cadherin EGF LAG seven-pass G-type receptor 3) was expressed in primary HBEs and in HBE cell lines, and its expression was downregulated in CF (F508del/F508del)-HBEs as compared to non-CF cells. CELSR3 belongs to the G-Protein Coupled Receptor (GPCR) protein family and to the adhesion GPCR subfamily. These particular GPCRs are characterized by a very long extracellular N-terminus containing among others several cadherin domains suspected to establish homophilic interactions between neighboring cells to mediate cell-cell interactions. To confirm the role of CELSR3 in epithelial polarity, we induced epithelial mesenchymal transition (EMT) in 16HBE monolayers with Transforming Growth Factor beta (TGF β) or cisplatin (CIS). Both molecules induced concomitant loss of CELSR3 and epithelial marker ZO-1 and gain of fibrotic markers vimentin and fibronectin. We also demonstrated that knocking down expression of CELSR3 in 16HBE cells enhanced TGF β -induced EMT. However, knocking-down CELSR3 did not modulate transepithelial electric resistance (TEER) i.e. epithelial barrier function. showing that CELSR3 is an epithelial marker but not a direct determinant of intercellular junction tightness.

Taken together, our results demonstrated that CELSR3 is an epithelial marker down-regulated in cystic fibrosis bronchial epithelial cells. Moreover, loss of CELSR3 function may confer profibrotic phenotype to CF cells.

P45

S3.5 Imbalance of mucus homeostasis in chronic pancreatitis

Anita Balázs¹, Julia Duerr², Zhe Zhou-Schuckow², Jolanthe Schatterny², László Tiszlavicz³, István Németh⁴, Balázs Kui¹, Zoltán Rakonczay¹, Marcus Mall², Péter Hegyi¹

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Introduction: Qualitative and/or quantitative changes of mucus in the pancreas can be an important factor both in the initiation and progression of chronic pancreatitis (CP)¹. Imbalance in mucus homeostasis can lead to elevation of fluid viscosity in the ductal lumen which can result slower secretion or mucoprotein plug formation within the pancreatic ducts². Therefore, our main objectives were to (i) investigate the mucus content both in human CP and experimental pancreatitis models and (ii) to correlate the mucus changes with ductal fluid secretion and pancreatic fibrosis.

Methods: Human and mouse pancreata were investigated. Human CP tissue samples were collected from surgically resected pancreata, whereas CP was induced by administration of 6x50µg/kg cerulein 3 times a week for 4 weeks period in mice. Morphometric analysis of mucus was carried out by CellF software. Total RNA was isolated from human and mouse tissue. The mRNA levels of different mucin subtypes were analysed by quantitative RT-PCR. Fluid secretion into the closed luminal space of the cultured pancreatic ducts was analysed using a swelling method, whereas the level of fibrosis was determined by trichrome staining.

Results: The mucus volume density ($V_{d_{muc}}$) of human PDEC was significantly higher in CP than in controls, in case of smaller ducts (ductal diameter < 100µm: $1.21 \pm 0.13 \text{ nl/mm}^2$ and $0.37 \pm 0.05 \text{ nl/mm}^2$, respectively). Similarly, mouse PDEC showed significantly higher $V_{d_{muc}}$ in CP than in controls, especially in ducts with smaller diameter (ductal diameter < 80µm: $0.72 \pm 0.06 \text{ nl/mm}^2$ vs. $0.005 \pm 0.0002 \text{ nl/mm}^2$, ductal diameter > 80µm: $0.075 \pm 0.020 \text{ nl/mm}^2$ vs. $0.016 \pm 0.004 \text{ nl/mm}^2$). Mucin gene expression analysis showed, that muc6 was ~1000-fold upregulated in mouse and 17-fold upregulated in human CP. Changes in the relative luminal volume of isolated pancreatic ducts from cerulein treated mice show an abolished basal and stimulated fluid secretion after 4 weeks of treatment. Analyses of the time course changes of mucin, fibrosis and ductal secretion during the first four weeks showed clear association between the elevation of mucus content, decrease in fluid secretion and accretion of fibrosis.

Conclusion: There is a clear quantitative and qualitative changes in mucus homeostasis during the development of CP. Our data suggest that these changes are in association with decreased fluid secretion and increased fibroses, however, the cause-and-effect relationship needs to be determined. This study was supported by MTA, OTKA, TAMOP and EPC fellowship.

1. Sarles, H. *et al.* Observations on 205 confirmed cases of acute pancreatitis, recurring pancreatitis, and chronic pancreatitis. *Gut* (1965).
2. Hegyi, P. & Petersen, O. H. The exocrine pancreas: the acinar-ductal tango in physiology and pathophysiology. *Rev. Physiol. Biochem. Pharmacol.* (2013).

P25

S3.6 Differential gene activation in normal and cystic fibrosis human bronchial epithelial cells upon *Pseudomonas aeruginosa* infection

Viviane Balloy^{1,2}, Soumaya Boudaya^{1,2}, Jean Yves Coppee³, Marie Agnès Dillies³, Hugo Varet³, Caroline Proux³, Harriet Corvol^{1,2,4}, Michel Chignard^{1,2}, Loïc Guillot^{1,2}

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Rationale: Cystic fibrosis (CF), a genetic disease caused by mutations in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene, is characterized by airway colonization by *Pseudomonas aeruginosa* (*P.a*) resulting in a prolonged inflammatory response. Airway epithelium plays an important role in the innate defense by sensing microorganisms that leads to the synthesis of chemokines and cytokines that orchestrate leucocytes recruitment and activation and secretion of antimicrobial peptides. In the present study, we performed transcriptomic analysis of *P.a*-infected primary bronchial epithelial cells from healthy donors and CF patients. The comparative analysis of mRNA expression profiles between CF and non-CF cells may help to understand how *P.a* differentially initiates infection and to explain its persistence in lungs in spite of a strong inflammatory response.

Methods: Human primary bronchial epithelial cells expressing a functional (non-CF) or mutated (CF) CFTR (F508Del homozygous) were infected by *P.a* (PAK strain) at 0.25 MOI for 0, 2, 4 and 6 hours. For each time point, biological quadruplicates (4 donors per group, non-CF and CF) were processed for mRNA profiling (a total of 32 samples) using Illumina mRNA sequencing. Statistical analysis provided lists of genes having a significant differential expression ($p < 0.001$) between the CF and non-CF at 0, 2, 4 and 6 hours of infection.

Results: We found 310, 295, 252 and 448 genes differentially down-regulated and 541, 343, 415, 532 differentially up-regulated in CF compared with non-CF cells at 0, 2, 4 and 6 hours of infection, respectively.

We identified differentially up-regulated genes with biological plausibility that could explain the intense inflammatory response and differentially down-regulated genes whose reduced expression of products could explain the development of chronic infection and the absence of anti-inflammatory response of infected CF patients. Several genes such as IL-6, IL-17C, KLF2, TLR4, SLPI or GSTT1 were already known to play a role in the epithelium-*P.a* interaction or in CF but interestingly some of the dysregulated genes were never or sparsely described in the lung CF disease.

Conclusions: The link between the worsening in CF and infection with *P.a* is well established. Different clinical approaches are used to eliminate *P.a* especially antibiotic prescriptions, but none of them leading to complete eradication. Here, we identified mRNA pattern associated with *P.a* infection that could explain the lack of *P.a* eradication in CF. This transcriptomic analysis may be the first step to study the development of new diagnostic tools and therapeutic strategies.

Funding: Vaincre la mucoviscidose

26 March — 18:00–19:00
Flash Paper Session

Transcription factors and miRNAs that regulate fetal to adult CFTR expression change are new targets for cystic fibrosis

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The CFTR gene displays a tightly regulated tissue-specific and temporal expression. Mutations in this gene cause cystic fibrosis (CF). In this study we wanted to identify trans-regulatory elements responsible for CFTR differential expression in fetal and adult lung, and to determine the importance of inhibitory motifs in the CFTR-39UTR with the aim of developing new tools for the correction of disease-causing mutations within CFTR. We show that lung development-specific transcription factors (FOXA, C/EBP) and microRNAs (miR-101, miR-145, miR-384) regulate the switch from strong fetal to very low CFTR expression after birth. By using miRNome profiling and gene reporter assays, we found that miR-101 and miR-145 are specifically upregulated in adult lung and that miR-101 directly acts on its cognate site in the CFTR-39UTR in combination with an overlapping AU-rich element. We then designed miRNA-binding blocker oligonucleotides (MBBOs) to prevent binding of several miRNAs to the CFTR-39UTR and tested them in primary human nasal epithelial cells from healthy individuals and CF patients carrying the p.Phe508del CFTR mutation. These MBBOs rescued CFTR channel activity by increasing CFTR mRNA and protein levels. Our data offer new understanding of the control of the CFTR gene regulation and new putative correctors for cystic fibrosis.

Full reference of the paper:

Eur Respir J. 2015 Jan;45(1):116-28. doi: 10.1183/09031936.00113214. Epub 2014 Sep 3.

MiR-31 Dysregulation in Cystic Fibrosis Airways Contributes to Increased Pulmonary Cathepsin S Production.

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Rationale: Cathepsin S (CTSS) activity is increased in bronchoalveolar lavage (BAL) fluid from cystic fibrosis (CF) patients. This activity contributes to lung inflammation via degradation of antimicrobial proteins such as lactoferrin and members of the α -defensin family. Objectives: In this study, we investigated the hypothesis that airway epithelial cells are a source of CTSS, and mechanisms underlying CTSS expression in the CF lung. Methods: Protease activity was determined using fluorogenic activity assays. Protein and mRNA expression were analysed by ELISA, Western blotting and RT-PCR. Main Results: In contrast to neutrophil elastase, CTSS activity was detectable in 100% of CF BAL fluid samples from patients without *Pseudomonas aeruginosa* infection. In this study, we identified epithelial cells as a source of pulmonary CTSS activity with the demonstration that CF airway epithelial cells express and secrete significantly more CTSS than non-CF controls in the absence of pro-inflammatory stimulation. Furthermore, levels of the transcription factor IRF-1 correlated with increased levels of its target gene CTSS. We discovered that miR-31, which is decreased in the CF airways, regulates IRF-1 in CF epithelial cells. Treating CF bronchial epithelial cells with a miR-31 mimic decreased IRF-1 protein levels with concomitant knockdown of CTSS expression and secretion. Conclusions: The miR-31/IRF-1/CTSS pathway may play a functional role in the pathogenesis of CF lung disease and may open up new avenues for exploration in the search for an effective therapeutic target.

Full reference of the paper:

Weldon S, McNally P, McAuley DF, Oglesby IK, Wohlford-Lenane CL, Bartlett JA, Scott CJ, McElvaney NG, Greene CM, McCray PB Jr, Taggart CC. miR-31 dysregulation in cystic fibrosis airways contributes to increased pulmonary cathepsin S production. *Am J Respir Crit Care Med.* 2014 Jul 15;190(2):165-74. doi:10.1164/rccm.201311-1986OC. PubMed PMID: 24940638; PubMed Central PMCID:PMC4226050.

Impaired TLR4 and HIF expression in cystic fibrosis bronchial epithelial cells downregulates hemeoxygenase-1 and alters iron homeostasis in vitro.

Shashi Chillappagari^{1,2,3,4}, Shalini Venkatesan^{5,3,4}, Virajith Garapati^{1,2,3,4}, Poornima Mahavadi^{5,3,4}, Antje Munder^{6,7}, Andreas Seubert⁸, Gaurav Sarode^{1,3}, Andreas Guenther^{5,9,3,4}, Bernd T. Schmeck^{2,3,4}, Burkhard Tümmler^{6,7}, Markus O Henke^{1,3,4,10,11}

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Hemeoxygenase-1 (HO-1), an inducible heat shock protein, is upregulated in response to multiple cellular insults via oxidative stress, lipopolysaccharides (LPS) and hypoxia. In this study, we investigated in vitro the role of toll-like receptor 4 (TLR4), hypoxia-inducible factor-1 α (HIF-1 α) and iron on HO-1 expression in cystic fibrosis (CF). Immunohistochemical analysis of TLR4, HO-1, ferritin and HIF-1 α were performed on lung sections of CFTR^{-/-} and wildtype mice. CFBE41o⁻ and 16HBE14o⁻ cell lines were employed for in vitro analysis via immunoblotting, immunofluorescence, real-time PCR, luciferase reporter gene analysis and iron quantification. We observed a reduced TLR4, HIF-1 α , HO-1, and ferritin in CFBE41o⁻ cell line and CF mice. Knockdown studies using TLR4-siRNA in 16HBE14o⁻ revealed significant decrease of HO-1, confirming the role of TLR4 in HO-1 downregulation. Inhibition of HO-1 using tin protoporphyrin in 16HBE14o⁻ cells resulted in increased iron levels suggesting a probable role of HO-1 in iron accumulation. Additionally, sequestration of excess iron using iron chelators resulted in increased HRE response in CFBE41o⁻ and 16HBE14o⁻ implicating a role of iron in HIF-1 α stabilization and HO-1. To conclude, our in vitro results demonstrate that multiple regulatory factors such as impaired TLR4 surface expression, increased intracellular iron and decreased HIF-1 α downregulates HO-1 expression in CFBE41o⁻ cells.

Full reference of the paper: Impaired TLR4 and HIF expression in cystic fibrosis bronchial epithelial cells downregulates hemeoxygenase-1 and alters iron homeostasis in vitro.

Chillappagari S, Venkatesan S, Garapati V, Mahavadi P, Munder A, Seubert A, Sarode G, Guenther A, Schmeck BT, Tümmler B, Henke MO.

Am J Physiol Lung Cell Mol Physiol. 2014 Nov 15;307(10):L791-9. doi: 10.1152/ajplung.00167.2014. Epub 2014 Sep 19.

Restoration of CFTR function in patients with cystic fibrosis carrying the CFTR F508del mutation

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Restoration of BECN1/beclin 1-dependent autophagy and depletion of SQSTM1/p62 by genetic manipulation or autophagy-stimulatory proteostasis regulators, such as cystamine, have positive effects on mouse models of human cystic fibrosis (CF). These measures rescue the functional expression of the most frequent pathogenic CFTR mutant, F508del, at the respiratory epithelial surface and reduce lung inflammation in F508del-CFTR homozygous mice(1,2). Cysteamine, the reduced form of cystamine, is an FDA approved drug. Here, we report that oral treatment with cysteamine greatly reduces the juvenile overmortality of CfrF508del mice fed with a normal diet from 49% to 9% (Log-Rank test, $p = 0.0001$) and improves the phenotype of newborn mice bearing the F508del-CFTR mutation. Cysteamine is also able to increase the plasma membrane expression of the F508del-CFTR Band C in freshly isolated nasal epithelial cells collected from F508del homozygous CF patients, and these effects persist for 24 h after cysteamine withdrawal. Importantly, this cysteamine effect after washout is further sustained up to 48 h by the administration of epigallocatechin gallate (EGCG), a green tea flavonoid, also endowed with the capability of inhibiting the activity of the protein kinase CK2, which favours CFTR fragmentation. Driven by these pre-clinical evidences, we performed a pilot clinical trial involving 10 F508del-CFTR homozygous CF patients (EudraCT number #2013-001258-82 approved by Local Ethical Committee, protocol reference #85/13), administered with the combination of cysteamine bitartrate (trade name Cystagon(r), Orphan Europe) at the same doses recommended for patients with cystinosis (FDA number NDA020392) (150 to 300 mg of cysteamine base every 6 h), and the over-the-counter food additive EGCG (trade name Epinerve(r), SIFI Pharmaceuticals, 270 mg once daily). We found that such a treatment is effective in restoring BECN1, reducing SQSTM1 levels and improving CFTR function up to >25% of the values of non-CF controls ($p < 0.001$) in nasal epithelial cells in vivo. These findings correlate (Spearman's $r = -0.883$, $p = 0.001$) with a decrease of chloride concentrations in sweat by >25% of baseline, with values falling below 60 mmol/L in 7 out of 10 patients. The treatment also induces a significant reduction of the abundance of TNF/TNF-alpha (tumor necrosis factor alpha) and CXCL8 (chemokine (C-X-C motif) ligand 8) transcripts in nasal brushing and TNF-alpha and CXCL8 protein levels in the sputum. Altogether, these results suggest that optimal schedules of cysteamine plus EGCG might be used for the treatment of CF caused by the CFTR F508del mutation.

References: 1) Luciani A, et al. Nat Cell Biol 2010; 12:863-75. 2) Luciani A, et al. Autophagy 2012;8:1657-72.

Full reference of the paper: Restoration of CFTR function in patients with cystic fibrosis carrying the F508del-CFTR mutation. De Stefano D, Vilella VR, Esposito S, Tosco A, Sepe A, De Gregorio F, Salvadori L, Grassia R, Leone CA, De Rosa G, Maiuri MC, Pettoello-Mantovani M, Guido S, Bossi A, Zolin A, Venerando A, Pinna LA, Mehta A, Bona G, Kroemer G, Maiuri L, Raia V. Autophagy. 2014 Nov 2;10(11):2053-74. doi: 10.4161/15548627.2014.973737.

27 March — 08:45–10:30

Symposium 4 - Host-pathogen interaction

S4.1 Modulation of the host immunity by *Pseudomonas aeruginosa* and *Staphylococcus aureus*: impact on the pulmonary microbiome

Erwan Pernet^{1,2}, Jérémy Brunet¹, Laurent Guillemot¹, Michel Chignard¹, Yongzheng Wu¹, Lhousseine Touqui¹

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In the lungs of CF patients, alterations of CFTR functions cause depletion of airway surface liquid and mucus dehydration, which provide appropriate niche for chronic bacterial infection by opportunistic pathogens. Bacterial airways infection varies significantly with the age of patients indicating the pulmonary microbiota of CF patients is a dynamic process. However, the mechanisms responsible for this age-related infection changes remained unclear. The type-IIA secreted phospholipase A2 (sPLA2-IIA) is a highly bactericidal enzyme produced by bronchial epithelial cells (BECs) and alveolar macrophages (AMs). In our previous works, we have shown that sPLA2-IIA is present in the expectorations of CF patients and that its levels increased with the age of these patients. *Pseudomonas aeruginosa* induces type-IIA secreted phospholipase A2 production by BECs and AMs and that this enzyme contributes to the eradication of Gram-positive bacteria such as *Staphylococcus aureus*. Our studies suggested that sPLA2-IIA can play a role in the modulation of CF airways microbial communities. In the present work we investigate the effect of *S. aureus* on the expression of sPLA2-IIA and the consequence on the survival of this bacterium. *S. aureus* can secrete immune modulatory molecules to dampen the host immunity and the recently identified adenosine is one of these molecules. But the role of adenosine in sPLA2-IIA is still unknown. Using a guinea pig model of pulmonary infection by this bacterium, we showed the *S. aureus* strain lacking adenosine production system increases sPLA2-IIA expression in AMs. This was accompanied by increased airways clearance****of *S. aureus*. Peptidoglycan of *S. aureus* was identified to induce sPLA2-IIA expression in AMs *via* NOD2. This intracellular receptor increases the phagocytosis of *S. aureus* and its subsequent killing by sPLA2-IIA. In contrast, *S. aureus* adenosine down-regulates this phagocytosis-bridged process of sPLA2-IIA expression by AMs. This inhibition involves adenosine receptors A2a, A2b and a PKA-dependent pathway. Our studies suggest that *S. aureus* escapes sPLA2-IIA-mediated killing from the airways through adenosine-mediated inhibition of phagocytosis and sPLA2-IIA expression. Therefore, sPLA2-IIA is an actor that can modulate microbial communities composition in CF airways.

S4.2 Host-pathogen interactions in CF lung disease

Dominik Hartl

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CF patients are polymicrobially colonized with bacteria and fungi. Since this colonization is chronic, the host-pathogen interaction is dynamic and evolving over time. The first line of host defence represent resident epithelial cells (producing anti-microbial peptides) and recruited phagocytes (neutrophils and macrophages). The second, and more sustained, immune response is characterized by an augmented Th2 and Th17 and a dysregulated Treg response. The precise mechanisms underlying this immune skewing are only partially understood, but probably involve both CFTR-dependent effects and chronic infections. In my talk, I will focus on the interaction of *Pseudomonas aeruginosa* with immune cells. Understanding host-pathogen interactions is essential to develop novel immune-based therapeutic strategies for CF lung disease in the future.

S4.3 Normalisation of inflammatory responses in CF through induction of the NF-kappaB regulator A20

Bettina C Schock, Madeleine Ennis, Shu-Dong Zhang

Queen's University Belfast

In Cystic Fibrosis (CF) airway disease dehydrated epithelial surfaces, 'sticky' mucus, defective mucociliary clearance and chronic bacterial colonisation lead to altered activation of the innate immune system.

We describe persistent surface and cytoplasmic expression of Toll-Like Receptor 4 (TLR4) in CF epithelial cells and investigations of lysosomal markers suggest an inability of these cells to target TLR4 towards the lysosome for degradation.

Additionally, after TLR4-LPS binding, NF-kB activation is not terminated in a timely fashion, leading to increased production of pro-inflammatory cytokines. Recent work focuses on the negative NF-kB regulator TNFalpha induced protein 3 (TNFAIP3, A20). In the airways, A20 is normally upregulated in response to infection, but in CF airways A20 induction (mRNA) is delayed and functional protein is reduced, contributing to the hyper-inflammatory phenotype in CF airway epithelial cells. As a proof of principle we show here that pharmacological induction of A20 with the plant diterpenoid gibberellin (GA₃) has anti-inflammatory effects and we have now used an advanced bioinformatics approach (connectivity mapping) to predict drugs already licensed for the use in humans that should be able to induce A20 and thereby normalise the inflammatory response. Drug repositioning should be able to provide CF patients with alternative anti-inflammatory treatments faster than conventional drug development. The action of the predicted drugs on A20 levels, p65 expression, IL-8 release etc. will be described.

Finally increased A20 repression and other pro-inflammatory signalling pathways might also contribute to the lack of timely regulation of inflammatory signalling and such findings will be discussed.

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S4.4 Dissection of host susceptibility to *Pseudomonas aeruginosa* lung infection in mice

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The progression and severity of *P. aeruginosa* infection do not appear to correlate with the type of *CFTR* variant and rather seems to be largely dependent on other factors, which remain to be established. Generally, host susceptibility to infectious diseases including to *P. aeruginosa* are complex traits that need to be analyzed to understand the full repertoire of host response to this pathogen. To map host genes underlying such complex diseases, well-defined mouse genetic reference populations of inbred mice have been a powerful tool. As a first step towards establishing mouse mapping resource population, we assessed nine classical inbred strains of mice (A/J, BALB/cJ, BALB/cAnNCrI, BALB/cByJ, C3H/HeOuj, C57BL/6J, C57BL/6NCrI, DBA/2J, and 129S2/SvPasCRL) for the susceptibility to *P. aeruginosa* clinical strains infection. Recently, we demonstrated that the genetic makeup of the host influences the response to infection (De Simone *et al*, PlosOne 2014). In particular, A/J and C3H/HeOuj showed the most deviant clinical (e.g. body weight, mortality, chronic infection) and immunological phenotypes (e.g. cytokines response, innate and adaptive cell response). Based on these results, susceptible A/J (mean survival time of 0.5 days) and resistant C3H/HeOuj (mean survival time of 3.8 days) mice were crossed to generate 400 F2 population, and subsequently used to map host genetic determinants of susceptibility to *P. aeruginosa*. All F2 mice were challenged with *P. aeruginosa* clinical strain, and monitored for mean survival time up to seven days post infection, as disease phenotype associated trait. In particular, a significant different mean survival time ranging from 0.5 to 7.0 days was observed in F2 mice population compared to the parental lines. Thus, the phenotypic extremes (20% high susceptible and 20% most resistant) of F2 population were genotyped with about 10K informative single nucleotide polymorphic (SNP) markers, and subsequently performed quantitative trait loci (QTL) mapping. We identified a significant QTL on chromosome 6 at 90.8 Mbp with 4.3 LOD Score, with a 1.5-LOD drop support interval of 81.5-102.2 Mbp. A total of 327 genes within the mapped locus were identified from the public database ([http:// www.informatic.jax.org/](http://www.informatic.jax.org/)), and classified in predicted proteins, RNA genes, heritable phenotypic markers and non-coding RNA genes. Gene prioritization approach by specific computational tools (Endeavour program) and score analysis revealed few genes possibly involved in susceptibility to *P. aeruginosa* infection : *Dok1*, *Tacr1*, *Cd207*, *Clec4f4*, *Gp9*, *Foxp1*, *Gata2* and *Wnt7a*, principally involved in pathogen sensing, neutrophils and macrophages recruitment, inflammatory cytokines expression and epithelial alveolar turnover process. Further characterization of these genes may lead to the identification of novel modifiers involved in the pathogenesis of CF and may complement human studies.

De Simone, M., L. Spagnuolo, et al. (2014). "Host genetic background influences the response to the opportunistic *Pseudomonas aeruginosa* infection altering cell-mediated immunity and bacterial replication." PloS one 9(9): e106873.

S4.5 A new synthetic compound inhibits the expression of Quorum-Sensing regulated genes and biofilm formation in *Pseudomonas aeruginosa*

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Objectives: *Pseudomonas aeruginosa* is a ubiquitous bacterium that can cause severe and chronic lung infections in patients with cystic fibrosis, particularly in establishing a structured form called biofilm, which is very difficult to eliminate by antibiotics. It has been shown that the bacterial Quorum-Sensing (QS) communication system play a key role in the development of biofilms and in orchestrating the expression of many *P. aeruginosa* virulence factors. *P. aeruginosa* possesses two complete QS systems, *rhl* and *las*, which are mainly controlled by two natural *N*-acyl homoserine lactones (HSL) signal molecules, C4-HSL and 3-oxo-C12-HSL respectively. Thus, the aim of our study is to design potent HSL analogs and to screen them for their antibiofilm activity and their effect on QS-regulated genes expression.

Methods: Various analogs were designed on the basis of the C4-HSL structure, by replacing the lactone ring, or the aliphatic chain, by groups modulating the electronic effects or the steric hindrance of the molecules. These compounds were synthesized and screened for their ability to impair biofilm formation in an innovative *in vitro* model developed in the laboratory. In this model, analogs effectiveness was evaluated by CFU enumerations of adherent cells and confocal microscopy observations. In parallel, the quantitative real-time RT-PCR was developed to analyze the effects of analogs on the expression of some QS-regulated genes.

Results: Among the analogs, a compound called C11 (N-pyrimidyl butanamide) showed a significant inhibition of biofilm formation in a dose-dependent manner, coupled with an absence of cytotoxicity on lung cells. C11 was then tested in combination with antibiotics and a significant synergistic antibiofilm effect was obtained with ciprofloxacin, tobramycin and colistin. Moreover, this inhibitory activity of C11/antibiotics combination was preserved on a biofilm developed under anaerobic conditions, set up to approximate the *in vivo* colonization conditions by the bacterium. Moreover, a significant down-regulation of QS-regulated genes by C11 was observed, both for genes regulating the *rhl* system (*rhlR* and *rhlI*) as the *las* system (*lasR* and *lasI*). After a structure-activity study performed on C11, new analogs were synthesized and three showed a significant antibiofilm activity.

Conclusion: The combination of C11 with antibiotics showed significant inhibitory effects on the biofilm development and the QS-regulated gene expression in *P. aeruginosa*. Different combination of analogs with antibiotics are actually tested in order to determine the best combination of effective molecules against the *P. aeruginosa* biofilm and to define optimal conditions for further *in vivo* investigations in a murine model of chronic lung infection.

This work is supported by Vaincre la Mucoviscidose and Association Gregory Lemarchal.

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S4.6 *Pseudomonas aeruginosa* adaptation to cystic fibrosis airways shapes the host response in mice during the progression of airway disease

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Background: Repeated cycles of infections, caused principally by *Pseudomonas aeruginosa*, combined with robust host immune response determine the course and outcome of respiratory disease in cystic fibrosis (CF). During the course of chronic colonization *P. aeruginosa* adapts to the airways and dramatically modifies its phenotype promoting evasion of the host immune recognition, as described previously. However, structural changes including degradation of elastin, collagen deposition and high level of glycosaminoglycans (GAG) and matrix metalloproteinases (MMP) are associated to the progression of lung disease in CF patients.

Objectives: It is still not clear whether and how the *P. aeruginosa* adaptation to CF airways affects the host immune response and favors airway damage and the remodeling processes leading to lung decline.

Method: For this study, we used a clonal lineage of *P. aeruginosa* strains sampled at the onset of infection and after 7.5 years before the patient died; they were selected for their diversity determined in previous characterization. These bacterial strains were challenged in C57Bl/6 and CF ko mice after their inclusion in agar beads to establish long-term chronic lung infection. Mice were evaluated for bacterial count, lung histopathology and markers of inflammation and tissue damage.

Results: Following the peak of infection, early *P. aeruginosa* strain embedded in agar-beads either disseminated systemically and induced death, or was cleared by the host, while CF-adapted variants persisted for up to three months in a set of mice (~30%) with a bacterial load stabilized at $\approx 10^4$ CFU/lung. Observing the murine host response over the course of the airways infection, CF-adapted variants resulted in lower recruitment of innate immune cells and production of cytokines/chemokines involved in leukocytes recruitments (day 28) compared to the early phase (day 2), while levels of IL-17 and IFN- γ , associated to lymphocyte polarization, remained high. The long-term consequence of CF-adapted variants is the implication of an adaptive immune response and the formation of bronchus-associated lymphoid tissue (BALT)-like structures, as indicated by histological scores. Immunohistochemistry with anti-CD3 and B220 antibodies showed that BALT structures were consisted mainly of T and B cells, confirming a shift in the subtypes of immune cells recruited during the course of chronic infection. Hallmarks of airway remodelling and fibrosis, including epithelial hyperplasia and structure degeneration, goblet cell metaplasia, collagen deposition and elastin degradation were also observed by the pathological scores. MMP-9 activity and protein, TGF- β and sGAG were detected and increased over time during the long-term infection in murine lungs infected with the CF-adapted variants. When long-term chronic infection was established in CF mice, we found higher goblet cell metaplasia in comparison to wt mice, but no differences for other markers.

Conclusions: We conclude that *P. aeruginosa* adaptation plays a significant role in the progression of airway disease shifting the host response toward airway remodeling and tissue damage. In addition, CF-adapted variants are critical success determinant for reproducing the human disease in mice.

Supported by Italian Cystic Fibrosis Research Foundation

27 March — 11:00–12:45

Symposium 5 - Mucus in lung health

S5.1 Mucociliary Interactions in Health and Disease

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The mucus clearance system of the lung represents a key innate immune system that protects the airway surface against constant exposure to inhaled infectious and noxious particles. Abnormal mucus clearance is an important contributor to the phenotype of patients with chronic bronchitis resulting from environmental and/or genetic causes. Increases in airway mucus concentration, as the result of reduced airway hydration and increased mucin secretion, appear to represent a unifying theme in both cystic fibrosis (CF) and COPD patients. However, the failure to regulate the hydration of the mucus layer, and its relationship to disease, is most apparent in CF, where dysregulation of CFTR combined with increased mucin production results in a reduction of both mucociliary clearance (MCC) and cough clearance (CC). While increases in mucus viscoelastic properties, as measured by classical rheological techniques, are the best-characterized properties of dehydrated/altere d mucus, there is currently a lack of knowledge regarding why such viscoelastic mucus reduces both MCC and CC rates. The overarching goal our work is to understand the biophysical basis of mucus stasis by identifying and measuring key parameters describing how changes in the mucus, mucus-cell surface interactions regulate mucus clearance. To this end, we have developed a novel description of mucus transport system, i.e., the "two-gel" mucus layer/periciliary layer (PCL), that emphasizes the role of the concentration of secreted mucins, i.e., their hydration, in the mucus layer to understand the efficacy of mucus clearance in health and disease. Based on this model, we believe that mucus dehydration increases the physical interactions between the mucus and the cell surface, thereby (1) increasing the friction imposed by cilia-mediated propulsion and (2) making harder to "disadhere" the mucus from the cell surface during cough. In this presentation, we will reveal how mucus dehydration, combined with alterations in mucus properties, produces adherent mucus that "sticks" to epithelial cells and produces in a slowing/failure in both cilia- and cough-mediated clearance mechanisms. In addition to the identification of new targets for the development of novel therapeutic agents to treat mucostasis, we believe that this model will aid in our ability to understand and predict when and why mucus clearance fails in disease.

S5.2 The respiratory mucus barrier; structure, assembly and intracellular packaging of the major gel-forming mucin, MUC5B

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The mucus barrier provides a dynamic, protective****interface between tissues and the outside environment. Innate defence mechanisms in the respiratory tract are centred on this barrier, which plays a key role in protection from infectious and environmental challenges¹. The components of mucus in terms of water, salts and in particular, secreted glycoproteins and proteins (at least 250 distinct molecular species)² come together to form a physical barrier armed with anti-microbial activity. Transport of mucus out of the airways by mucociliary clearance and cough is critical for health, and accumulation of mucus, with non-optimal transport properties, is a pathologic feature of airway diseases such as cystic fibrosis (CF) and chronic obstructive pulmonary disease (COPD). In CF, accumulation of mucus results in adherence of mucus to the airway epithelium, blocking small airways and providing an environment within which bacteria can flourish. This leads to infection, inflammation and a cycle of airway damage and mucus accumulation, key aspects of morbidity and mortality in this disease³.

The mucin glycoproteins are the major determinants of the physical properties of mucus in the healthy airways. These very large polymeric molecules are synthesized (and stored within secretory granules) by specialized secretory cells in the surface epithelium and submucosal glands and are secreted in response to a stimulus. The airway mucus gel is comprised of a heterogeneous mixture of two mucin glycoproteins, MUC5AC and MUC5B⁴. The roles of these two mucins are yet to be fully elucidated. MUC5B, the predominant mucin in health, is indispensable for mucociliary clearance that controls bacterial infection⁵. In contrast, MUC5AC is a more minor component of healthy mucus that is up-regulated during infection⁶ and allergic airway inflammation⁷.

The presentation will focus on new structural information on MUC5B, the molecular details of intracellular assembly and packaging of MUC5B into secretory granules and the post-secretory expansion of MUC5B to form mucus. The potential implications for mucus barrier properties will be discussed in relation to CF.

References

1. Thornton, D.J., Rousseau, K. & McGuckin, M. (2008)___Annu. Rev. Physiol. **70,** 5.1-5.28****
2. Kesimer,**M., Pickles, R.J., Henderson, A.G., Alexis, N.E.,**DeMaria, G., Knight, D., Kirkham, S., Thornton, D.J. & Sheehan, J.K. (2009)___Am. J Physiol. Lung Cell Physiol. (2009) **296,** L92-L100.****
3. Ehre, C., Ridley, C. and Thornton, D.J. (2014)___Int J Biochem Cell Biol. 52, 136-45.
4. Kirkham, S., Sheehan, J.K., Knight, D., Richardson, P.S. & Thornton, D.J. (2002)___Biochem J. **361,** 537-546.
5. Roy, M.G., Livraghi-Butrico, A., *et al.*, (2014) Nature **505 (7483)**, 412-6.
6. Hasnain, S.Z., Evans, C.M., Roy, M., Gallagher, A.L., Kindrachuk, K.N., Barron, L., Dickey, B.F., Wilson, M.S., Wynn, T.A., Grecnis, R.K. and Thornton, D.J. (2011) J. Exp. Med. **208(5)**, 893-900.
7. Young, H.W., O.W. Williams, *et al.* (2007) Am. J. Respir. Cell Mol. Biol. **37**, 273--290.

S5.3 Mucociliary Transport in Early CF

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CF is a multi-system disease caused by loss of the CFTR anion channel. Despite advances in many areas of CF research, patients with CF still experience significant morbidity, and CF remains a lethal disease. Although the early postnatal time seems central to the disease, this is precisely the time at which we know the least about the CF lung. With the establishment of universal newborn screening for CF, it is critically important to better understand disease pathogenesis at the very early time points in CF. However, these studies are difficult to perform in infants and young children. These early studies would be more feasible in a CF animal model that develops pulmonary disease similar to humans with CF. 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. Within several months CF pigs develop lung disease with features similar to human CF lung disease including bacterial infection, inflammation, mucus accumulation, airway remodeling, and airway obstruction. However, on the day they are born, CF piglet airways lack airway inflammation, goblet cell hyperplasia, submucosal gland hypertrophy, or mucus accumulation, yet already have a host defense defect. Mucociliary transport (MCT) guards the lung by trapping invading pathogens and particulates in mucus, which is then propelled up the airways by cilia. Although people with advanced CF can exhibit slowed MCT, whether MCT is impaired at the disease's origin has been unknown. MCT, assayed using an X-ray computed tomography-based approach to track discrete airway particles, appears similar in newborn CF and non-CF piglets under basal conditions. However, after cholinergic stimulation, which elicits copious mucus secretion from submucosal glands, many particles move normally in CF piglets, but some become stuck and fail to move up the airways. Subsequent mechanistic investigations using excised airways revealed that CF submucosal glands secrete strands and blobs of mucus that sometimes do not break free after emerging and remain tethered to the gland ducts, hindering MCT. Importantly, inhibiting anion secretion in non-CF airways replicates the CF abnormalities. These findings directly link impaired MCT to loss of CFTR anion transport, indicating that defective MCT is a primary abnormality not dependent on infection, inflammation or remodeling. Nevertheless, advancing infection and bronchiectasis might further disrupt MCT to fuel disease progression. These studies are increasing our understanding of the early events in the pathogenesis of CF lung disease and will hopefully lead to the development of new therapeutic tools for CF. Moreover, since defective CFTR function leads to disease in multiple organs, we expect that the mechanisms described here will be relevant for disease pathogenesis in other organs affected in CF.

S5.4 Defective PPARg signaling and increased mucin/bicarbonate expression in the regulation of human airway epithelial cell repair

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Cystic fibrosis (CF) is caused by mutations in the CFTR apical chloride channel which supports bicarbonate transport. Recurrent infection and inflammation of the CF airway epithelium causes continuous repair which may lead to remodeling of the normal pseudostratified mucociliated tissue. Direct cell-to-cell interactions are critical in coordinating repair and in the transition of repairing cells from a self-renewal, multipotent stage to an early differentiated stage. Although there is evidence that wound repair is defective in CF, the underlying mechanisms regulating this process are poorly known. Here, we monitored the gene expression changes of Notch and peroxisome proliferator-activated receptor g (PPARg) in a 3D human airway epithelium wound repair model. These pathways are involved in determining cell fate and specific lineages of undifferentiated progenitor epithelial cells. We have previously shown that the gap junction protein connexin26 (Cx26) is expressed in a cytokeratin14 (KRT14)-subpopulation of progenitor cells capable to re-differentiate into all three main cell types of the airway epithelium. Human airway epithelial cells (HAECs) from non-CF (NCF) and from CF patients were differentiated at the air/liquid interface. The cells were mechanically wounded and the expression and/or localization of transcription factors and Cxs were monitored by qPCR and immunofluorescence (IF) in repairing HAECs, 24hrs after wounding, when the ablated area was covered (wound closure; WC) and at 10, 20 and 30 days after WC. Hes1 gene expression was used to monitor the activation of Notch signaling pathway during repair. Hydroxyprostaglandin dehydrogenase 15-(NAD) (HPGD)- the enzyme providing PPARg's endogenous ligand 15-ketoprostaglandin E2 (15kPGE2)- expression was measured to follow PPARg pathway. Moreover, we measured the expression of MUC5AC, MUC5B and carbonic anhydrase2 and 12 (CA2 and CA12). Ciliated cell markers (FOXJ1 and DNAI1) transiently decreased during wound repair while markers of progenitor cells (KRT5 and KRT14) increased, validating our model. We found that Hes1 expression was unaltered during repair in CF cells as compared to NCF cells. In contrast, HPGD expression was lower in CF cells as compared to NCF cells ($p < 0.05$, $N=9/11$) in non-wounded (NW) cultures and after 30 days of repair ($p < 0.05$, $N=4/6$). Similarly, the expression of PPARg was also reduced 30 days after repair in CF cells as compared to NCF cells ($p < 0.05$, $N=4/6$). Interestingly, MUC5AC expression was reduced in CF NW cultures ($p < 0.05$, $N=6/4$). Moreover, MUC5B and CA2 expression, but not CA12, was significantly increased in repairing CF cells at 24hrs after wounding and at WC, as compared to NCF cells ($p < 0.05$, $N=4/6$). In both NCF and CF cells, wounding triggered progenitor cell proliferation and Cx26 expression increase, which returned to basal levels after WC. Cx26 plaque counts revealed by IF was markedly decreased upon PPARg activation with 15-kPGE2 while PPARg inhibition with its pharmacological inhibitor GW9662 increased Cx26 plaque counts ($p < 0.05$, $N=4$). Our data show a deregulation of PPARg pathway in CF cells that may affect the repair process by changing Cx26-mediated cell-cell interactions and mucus cell differentiation. Experiments are underway to determine the causal relationships between CFTR, PPARg signaling and mucin/bicarbonate production.

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S5.5 Structure activity relationship for OligoG-induced normalization of the CF mucus phenotype

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Objectives: Cystic fibrosis (CF) is a recessive genetic disease caused by nonfunctional chloride and bicarbonate ion transport via CFTR. In the lungs of these patients, dense, intractable mucus collects because it is insufficiently cleared by the mucociliary clearance system, leading to bacterial colonization, lung infections and tissue damage.

We have previously shown that the gel-forming MUC2 mucin is packed in an orderly way that allows unpacking by calcium chelation and increased pH (1). Using an explant system (2), we found that, in contrast to normal mucus, the mucus of the small intestine is attached to the epithelium in mice without a functional CFTR channel (CftrF508-del). This mucus could be reverted to a non-attached phenotype by apical solutions containing above 100 mM bicarbonate (3).

OligoG CF-5/20 (AlgiPharma, Sandvika, Norway) is a refined oligosaccharide mixture derived from polymeric sodium alginate comprising mainly guluronate and some mannuronate residues, with average length of 13 monomers. OligoG is currently in clinical trials as inhalation therapy in CF patients. We have now tested and compared OligoG and alginate subfractions with different composition on mouse ileum CF explants and recorded to what extent OligoG can affect the attached CF mucus.

Methods: Explants from the small intestine of Cftr508-del mutant mice were mounted in the horizontal Ussing-type chamber (2). Alginate at different concentrations or composition was added to the apical buffer, pH 7.4. The attachment of the already formed mucus was assessed by comparing the total mucus thickness before and after aspiration.

Results: OligoG at 1.5% or higher normalized the mucus phenotype without increase in mucus thickness. At 1% OligoG the mucus remained attached and at 1.2% an intermediate phenotype was observed. OligoG fractions containing an average of 12 guluronate units were most effective, whereas shorter and longer molecules were less efficient. Mannuronic polymers were inactive in this assay.

Conclusion: These results suggest that it is guluronic acid and its calcium chelating capacity that is the important and active component in OligoG. These observations indicate that OligoG could act by normalizing the mucus layers in both the intestine and potentially the lungs of cystic fibrosis patients at therapeutically relevant concentrations.

1. Ambort, D., Johansson, M. E. V., Gustafsson, J. K., Nilsson, H., Ermund, A., Johansson, B. R., Kock, P., Hebert, H., & Hansson, G. C. (2012) *Proc. Natl. Acad. Sci. U. S. A.***109**, 5645-5650.
2. Gustafsson, J. K., Ermund, A., Johansson, M. E. V., Schutte, A., Hansson, G. C., & Sjovall, H. (2012) *Am. J Physiol Gastrointest Liver Physiol.***302**, G430-G438.
3. Gustafsson, J. K., Ermund, A., Ambort, D., Johansson, M. E. V., Nilsson, H. E., Thorell, K., Hebert, H., Sjovall, H., & Hansson, G. C. (2012) *J. Exp. Med.***209**, 1263-1272.

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S5.6 The role of mucus and mucin in mediating *Pseudomonas aeruginosa* infection and virulence in cystic fibrosis

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Pseudomonas aeruginosa colonises the mucus in the lungs of individuals with cystic fibrosis (CF). The prevention of chronic *P. aeruginosa* infection is a cornerstone of clinical care in CF. In this study we hypothesise that the environment of the CF lung, which contains thick stagnant mucus and mucins with altered glycans compared to non diseased individuals plays an important role in initiation of colonisation and maintenance of chronic bacterial infection. To test this hypothesis we have characterised the interaction of *P. aeruginosa* isolates with a variety of respiratory mucus secreting and non mucus secreting cell lines. Binding of *P. aeruginosa* strain PAO1 and strains from CF and non CF patients to mucins purified from cell lines and from different human and animal anatomical sites was assessed using a novel mucin microarray platform. Neo-glycoconjugates printed on a microarray were also probed with *P. aeruginosa* strains in order to identify potential inhibitors of initial colonisation. The effect of mucus on biofilm formation and antibiotic sensitivity was also assessed.

Infection assays showed that while *P. aeruginosa* strains colonised mucus secreting and non-mucus secreting respiratory cells, it displayed a predilection for mucus on the surface of cells. In addition bacterial aggregates were observed in cells of CF and non CF origin that formed adherent mucus layers. Higher numbers of bacteria were found to colonise a CF mucus secreting cell line than colonised non CF mucus secreting cells.

Strong binding of CF *P. aeruginosa* isolates and strain PAO1 to mannose, glucose, galactose, fucose and to the blood group antigens Lewis^x, Lewis^b, and H2 was detected. Differences in binding to sialyl Lewis^x, Lewis^y and 3' and 6' sialyl lactose was observed between strains. Similar binding profiles were seen for strains grown to log and stationary phase. The strength of the interaction of *P. aeruginosa* with mucins was not as strong as that seen with individual glycans. Binding of *P. aeruginosa* to native mucins from human and animal sources depended on the bacterial strains and the source of mucin suggesting that strains express different mucin binding adhesins. In contrast strains exhibited a very similar binding profile to mucins from cell lines with less complex truncated mucins compared to native mucins.

P. aeruginosa exhibited strong binding to the human blood group antigen, Lacto-N-fucopentose I, and incubation of *P. aeruginosa* with Lacto-N fucopentose I inhibited bacterial binding to both mucus secreting and non-mucus secreting cells. Growth of *P. aeruginosa* in the presence of mucin promoted both biofilm formation and antibiotic resistance.

The reasons for the particular predilection of *P. aeruginosa* for the CF airway, and the very significant negative clinical implications of colonisation are incompletely understood. Together these results suggest that mucus promotes both infection and virulence of *P. aeruginosa*. The identification of both bacterial and host factors that mediate infection with *P. aeruginosa* in CF patients should lead to the development of new therapeutic strategies to prevent disease.

28 March — 08:45–10:30

Symposium 6 - CFTR modulators and pharmacological correction of cystic fibrosis

S6.1 Small molecule screens for CFTR modulators

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We have continued to develop and carry out high-throughput screens to identify small molecule modulators of wildtype CFTR function (inhibitors and activators), Δ F508 potentiators and correctors, and modulators of less common CF-causing CFTR mutants. CFTR inhibitors, the most potent being (R)-BPO-27 (IC₅₀ ~4 nM), are potentially useful to treat bacterial secretory diarrheas and polycystic kidney disease. Recent patch-clamp and modeling results suggest that the inhibition mechanism of (R)-BPO-27 involves competition with ATP binding at the CFTR canonical ATP binding site. New, nanomolar-potency activators of wildtype CFTR have been identified for potential therapy of dry eye, constipation and non-CF lung diseases. Following our identification of the first Δ F508 correctors in 2005, we have reported new paradigms for second-generation screening, including synergy screens, and identification of compounds with dual corrector-potentiator function. Our most recent 'third-generation' screens are CFTR substructure target-based, including cell-based screens for correctors of NBD1 folding, and phenotype screens using conditionally reprogrammed human airway Δ F508 epithelial cells. With regard to potentiators, we recently identified novel potentiators with low nM potency that, unlike VX-770, do not reduce corrector efficacy or destabilize Δ F508-CFTR. Finally, screens have been established for a specific CF-causing mutation, W1282X, in which truncated and full-length (albeit mutated) CFTR can be generated, focusing on mutation-specific correctors, potentiators and read-through agents. Though drug development is best done commercially, academic discovery efforts remain useful to establish novel screening paradigms and mechanisms, and for personalized approaches to rare CFTR mutations.

S6.2 CFTR modulators for treatment of Cystic Fibrosis

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To address the effects leading to cystic fibrosis, two biomolecular activities are required, namely correctors to increase CFTR levels at the cell surface and potentiators which allow the effective opening of the CFTR channel. Combined, these activities allow chloride ion transport yielding improved hydration of the lung surface.

Research activities have identified several compounds capable of performing both these roles individually. Herein we report the identification of a novel, highly potent series of potentiators as well as our progress on the identification of corrector molecules which restore >50% of WT current in F508del homozygous primary cell cultures, when combined with our lead potentiator, GLPG1837.

The identification of GLPG1837 was through a YFP halide assay screen using low temperature-rescued F508del CFTR in CFBe41o- cells, which resulted in a number of compounds able to open the channel with potencies around 1 μ M. Optimization of one of the series identified led to improvements in the potency down to <10nM for a number of leads. In Ussing chamber tests, multiple compounds showed strong and dose-responsive activity, both on VX-809 rescued F508del homozygous and G551D/F508del heterozygous primary cells. The results on F508del homozygous primary cells were replicated in TransEpithelial Clamp Circuit (TECC) equipment, with strong signals for the active compounds.

Novel corrector molecules were identified from HTS screens on CFBe41o- cells harboring F508del CFTR. Several chemically diverse series were identified and were further developed by medicinal chemistry. Corrector compounds are available that exhibit channel opening activity in primary cells derived from F508del CFTR homozygous patients as measured by TECC. Combination of several novel corrector hits with C18 and GLPG1837 in primary F508del homozygous HBE cells, yielded >50% of WT CFTR correction.

In summary, we have developed a novel potentiator series with improvements in channel opening activity. While GLPG1837, our lead potentiator identified from the series, is currently in Phase I trial, we are actively optimizing several corrector series in preclinical stages which, when combined with our potentiators and C18, lead to a restoration of at least 50% of WT CFTR current in TECC assays.

S6.3 Challenges in Pharmacological Correction of Mutant CFTR

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The development of drugs that directly affect CFTR function, including the potentiator ivacaftor (VX-770, trade name Kalydeco) and the corrector lumacaftor (VX-809), offers an exciting new approach to pharmacological treatment of cystic fibrosis (CF). VX-770 potentiates CFTR function in gating mutations such as G551D in human bronchial epithelial (HBE) cells and results in robust clinical improvement in patients with this mutation. However, while short-term studies suggested that combining VX-809 and VX-770 could functionally correct $\Delta F508$ CFTR, the clinical response in $\Delta F508/\Delta F508$ patients was less impressive. We found that acute and chronic VX-770 treatment improved CFTR function in G551D cultures, consistent with clinical studies. However, our studies with $\Delta F508/\Delta F508$ HBE cultures chronically treated with VX-809 and VX-770 showed a decrease in CFTR function reflected by the destabilization of corrected $\Delta F508$ CFTR by VX-770. Thus, chronic treatment with CFTR potentiators and correctors may have unexpected effects that cannot be predicted from short-term studies and may require careful monitoring of dosing or development of new potentiators that do not interfere with CFTR stability. HBE cells from patients carrying $\Delta F508$ and the missense mutation R117H showed benefit from chronic VX-809 or VX-770, and the combination of these two compounds resulted in the largest CFTR-mediated responses. However, CF HBE bearing another missense CFTR mutation, N1303K, did not show marked rescue with VX-809 or VX-770. CFTR truncations such as G542X and W1282X did not result in production of CFTR protein. These findings and the observation that patients with similar CFTR mutations may respond differently to drugs, suggest that personalized treatment strategies that target specific mutations in individuals are necessary. Because use of primary CF HBE with specific mutations is limited by acquisition of donated lungs, we used conditionally reprogrammed cells (CRC) (Liu et al. 2012 Am J Pathol) to expand supplies of HBE and human nasal epithelial cells with defined CFTR mutations. CRC cultures retained CFTR function over multiple passages, allowing for the investigation of personalized, genotype-specific rescue of mutant CFTR by small-molecule therapies. We are also assessing CFTR function in a physiologically relevant ex vivo assay through the use of 3D organoids readily prepared from bronchial and nasal tissues. These bronchospheres and nasospheres allow detection of acute volume changes in response to CFTR activation. After inhibition of ENaC, the forskolin-induced luminal ion and fluid secretion is completely CFTR dependent and can be accurately quantified by measurement of organoid swelling using live-cell imaging. The ultimate goal is to utilize CRC and organoid approaches to test different combinations of potentiators and correctors to develop the optimal treatment strategy for each CF patient.

Supported by CFF, NIH, and Else Kröner-Fresenius-Stiftung.

S6.4 Analysis of the chronic effects of lumacaftor and ivacaftor on F508del-CFTR single-channel gating and stability

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Combination therapy with cystic fibrosis transmembrane conductance regulator (CFTR) correctors and potentiators is required to rescue F508del-CFTR, the most common cystic fibrosis (CF) mutation. In recent clinical trials, chronic co-administration of the corrector lumacaftor (VX-809) and the potentiator ivacaftor (VX-770) produced a small, but significant improvement in lung function and improved disease stability¹. However, other studies^{2,3} found that chronic co-administration of lumacaftor and ivacaftor diminishes F508del-CFTR function *in vitro*. In this study, we investigated, at the single-channel level, the effects of chronic co-administration of lumacaftor and ivacaftor on F508del-CFTR using BHK cells expressing F508del-CFTR (F508del-CFTR-BHK cells). We evaluated F508del-CFTR plasma membrane stability at 37 °C by normalizing open probability (P_o) values to those initially recorded at 37 °C after full channel activation (% P_o ; Ref 4).

After chronic treatment with lumacaftor (3 μ M for 24 h at 37 °C), F508del-CFTR channel gating was similar to that of low temperature-rescued F508del-CFTR Cl⁻ channels (P_o ~0.1; n = 7–10) and characterised by a severe gating defect with brief channel openings separated by prolonged channel closures. There was also no difference in plasma membrane stability (% P_o @9 min, ~0%; n = 7–10).

Acute treatment with ivacaftor (10 μ M) restored wild-type levels of P_o to either chronic lumacaftor-treated or low temperature-rescued F508del-CFTR Cl⁻ channels (P_o ~0.45; n = 5–6). The plasma membrane stability of these low temperature-rescued F508del-CFTR channels was worse, with P_o declining to zero quicker than in the absence of ivacaftor. However, there was a small improvement in the plasma membrane stability of the chronic lumacaftor-treated F508del-CFTR channels acutely treated with ivacaftor (% P_o @9 min, 16%; n = 4).

To investigate the impact of chronic co-incubation with lumacaftor and ivacaftor on F508del-CFTR Cl⁻ channels, we incubated F508del-CFTR-BHK cells with lumacaftor (3 μ M) and ivacaftor (1 μ M) for 24 h at 37 °C. Upon activation with PKA and ATP, the single-channel activity of F508del-CFTR was increased greatly (P_o ~0.58; n = 10) and characterised by frequent long channel openings. Of note, the plasma membrane stability of these highly active F508del-CFTR Cl⁻ channels was enhanced markedly

(% P_o @9 min, 67%; n = 8). To try to reconcile these results with previous findings¹⁻³, we studied F508del-CFTR Cl⁻ currents in excised membrane patches from F508del-CFTR-BHK cells chronically co-incubated with lumacaftor (3 μ M) and ivacaftor (1 μ M). Upon activation with PKA and ATP, we observed two populations of F508del-CFTR Cl⁻ current. Most F508del-CFTR current deactivated promptly, but the residual current exhibited delayed deactivation similar to the highly active long-lived F508del-CFTR Cl⁻ channels that we observed in single-channel recordings. We conclude that chronic co-incubation appears to rescue a sub-population of F508del-CFTR Cl⁻ channels. Future studies should identify and characterise these F508del-CFTR Cl⁻ channels.

Supported by the CF Trust; YW supported by Beijing Sun-Hope Intellectual Property Ltd.

References:

- [1] Boyle MP *et al.* Lancet Respir Med. 2014; 2:527-38.
- [2] Cholon DM *et al.* Sci Transl Med. 2014; 6:246ra96.
- [3] Veit G *et al.* Sci Transl Med. 2014; 6:246ra97.
- [4] Wang Y *et al.* Br J Pharmacol. 2014; 171:4490-503.

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S6.5 Task force for CF: an Italian drug discovery project to identify novel correctors and potentiators of F508del-CFTR

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Deletion of phenylalanine 508 (F508del), the most frequent mutation among patients with cystic fibrosis (CF), causes a defective maturation of CFTR protein. The maturation defect can be treated with chemical compounds known as correctors. F508del and other mutations like G551D also show a gating defect that requires another type of compounds called potentiators. At the moment there are no F508del correctors with adequate efficacy. Pharmacological rescue of F508del-CFTR function may also benefit from the identification of novel potentiators devoid of the negative effect shown by the new drug VX-770 and other potentiators in recent in vitro studies (Cholon et al., *Sci Transl Med* 6: 246ra96, 2014; Veit et al., *Sci Transl Med* 6: 246ra97, 2014). Indeed, it has been observed that some potentiators have an undesired activity on F508del-CFTR protein processing, resulting in decreased activity of VX-809 as corrector.

To identify drug-like small-molecules that rescue mutant CFTR function, we adopted a high-throughput screening approach. We screened a chemical library (selected according to stringent drug-likeness and maximal diversity criteria) containing 11,334 compounds using two different cell types (FRT and CFBE41o-) expressing F508del-CFTR. For the screening, we used two functional assays based on the halide-sensitive YFP, designed to identify correctors and potentiators. The screening identified:

- i) 104 hits active as potentiators among which 5 compounds with activity comparable to that of VX-770;
- ii) 5 new correctors with activity on both FRT and CFBE41o-cells.

All compounds have been confirmed and characterized with dose-response experiments and cytotoxicity tests.

The effect of selected correctors was analyzed by determining the electrophoretic mobility and the pattern of band B and band C. Some compounds increase both band C and band B, an effect that could be explained with decreased degradation of CFTR protein (or increased CFTR protein synthesis). Other compounds show a prevalent effect on band C which could suggest an improvement in protein stability/maturation. The specificity of correctors was confirmed by using the selective CFTR inhibitor 172 and by testing the correctors on parental cells which have negligible expression of F508del-CFTR.

All potentiators detected in the primary screening were tested at different concentrations on cells expressing F508del- and G551D-CFTR. Interestingly, many compounds are effective on both mutants with a strong increase in CFTR activity that in some cases approaches the effect of VX-770. In particular, one compound has high efficacy and an interesting submicromolar potency. To check whether chronic incubation with potentiators interferes with pharmacological rescue of F508del-CFTR by VX-809, we incubated the cells for 24 hours with compounds together with VX-809. We found that many potentiators indeed decrease the activity of F508del-CFTR. However, other compounds do not display this undesired activity, despite a significant potentiator activity. Such compounds could be considered for the development of potentiators selective for F508del.

The active compounds identified so far will be a starting point for the synthesis of novel potentiators and correctors having improved potency and efficacy.

S6.6 Mechanism-based screens lead to the identification of complementary pharmacological correctors of F508del CFTR

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The most prevalent form of Cystic Fibrosis is caused by F508del in CFTR. This mutation leads to misfolding of the nucleotide-binding domain 1 (NBD1) and perturbs normal interdomain interactions in the CFTR protein, resulting in the absence of functional CFTR chloride channels in the plasma membrane. Recent progress suggests targeting multiple steps in CFTR folding (e.g., NBD1 folding and interdomain interaction) may result in much more efficacious correction of CFTR F508del. However, the first generation of F508del correctors, which was discovered through phenotypic screening, appears to largely address just one aspect of the complex misfolding.

Harnessing the genetic clustering of suppressor mutations of F508del, we developed allelic screens with the goal to enrich for small molecules that preferentially modulate NBD1 folding or the interdomain interactions. A big proportion of F508del partial suppressors can be classified as NBD1 stabilizers or interdomain modifiers. Mutations from these two classes can complement to produce significantly more correction of F508del. The screening relies on CFBE410⁻ cells expressing CFTR F508del with partial suppressors of either NBD1 misfolding (3S) or interdomain perturbations (R1070W), and horseradish peroxidase (HRP) reporter is inserted into an extracellular loop of CFTR. Such a reporter allows a robust read-out for CFTR in the plasma membrane. Screening of more than 70,000 compounds at 10 μ M in both CFTR F508del 3S and F508del R1070W alleles resulted in different classes of hits. To profile these hits a mechanism-based screening index was developed in addition to the efficacy indices in both cell lines. The hits (>40% over vehicle) are characterized not only for the efficacy of F508del correction but also for the specific enrichment in either allele. The rates of both hit confirmation and translation into CFTR function (electrophysiology) and trafficking (Western analysis) are greater than 67%. Of these hits, about 15% have demonstrated corrector activity in primary hBE cells. Several hits could be expanded to early series with F508del corrector activity in primary CF hBE cells. The most advanced series to date reaches the efficacy level of VX-809 before any chemical optimization.

To target multiple steps in CFTR folding, corrector combination screening has been developed using randomized pairs of two correctors for which we measure the efficacy of rescuing the disease allele CFTR F508del. A single concentration was selected for each corrector based on its apparent EC₉₅ in primary CF hBE cells. A confirmation assay has also been developed in the form of dose response matrix of the corrector pair. The limited combination screening leads to the discovery of complementary corrector pairs that achieve significantly greater levels of F508del correction than VX-809 alone. Our approach is neither dependent on nor limited to any existing corrector. Hence it can be applied to any pharmacological development of F508del correction. Further application of the corrector combination screening will help to select compounds based on their potential for pharmacological complementation.

28 March — 11:00–12:30

Debate - Personalised Medicine for CF

28 March — 14:00–15:45

Symposium 7 - Protease-antiprotease imbalance in CF

S7.1 Elucidating in vivo roles of neutrophil elastase and other proteases in CF-like lung disease in mice

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Recent evidence from observational studies in infants with CF diagnosed by newborn screening suggests that neutrophil elastase (NE) is a key risk factor for the onset and progression of CF lung disease (Sly PD et al. *N Engl J Med* 2013). However, the role of NE and potentially other proteases in the complex in vivo pathogenesis of CF lung disease remains poorly understood. To elucidate the in vivo role of NE in the development of key features of CF lung disease including airway inflammation, mucus hypersecretion and obstruction, bacterial infection and structural lung damage, we used the bENaC-Tg mouse as a model of CF lung disease and determined effects of genetic deletion of NE on the pulmonary phenotype. In addition, we used unbiased expression profiling, genetic and pharmacological inhibition, Foerster resonance energy transfer (FRET)-based activity assays and genetic association studies to identify and validate other proteases implicated in lung disease in bENaC-Tg mice and patients with CF. In a series of experiments, we demonstrated that lack of NE significantly reduces airway neutrophilia, elevated mucin expression, goblet cell metaplasia and distal airspace enlargement, but has no effect on airway mucus plugging, bacterial infection or pulmonary mortality in bENaC-Tg mice (Gehrig S et al. *AJRCCM* 2014). Further, we identified macrophage elastase (matrix metalloproteinase 12; MMP12) as a highly up-regulated gene in lungs from bENaC-Tg mice and demonstrated that MMP12 contributes significantly to progressive emphysema formation, but has no effect on mucus obstruction and associated mortality. By using FRET reporters, we showed that MMP12 activity is elevated on the surface of airway macrophages from bENaC-Tg mice and patients with CF. Moreover, we found that a functional polymorphism in MMP12 (rs2276109) is associated with severity of lung disease in CF (Trojanek JB et al. *AJRCMB* 2014). Taken together, our results suggest that NE and MMP12 released from activated neutrophils and macrophages in mucostatic airways play important roles in the in vivo pathogenesis and may serve as potential therapeutic targets for early structural lung damage in CF. However, genetic deletion and pharmacological inhibition of NE and MMP12 did not prevent airway mucus plugging and associated mortality in bENaC-Tg mice suggesting that additional mukokinetic strategies (i.e. rehydration) may be required for effective treatment of airway mucus obstruction in CF.

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S7.2 Role of Host and *Pseudomonas* proteases in the modulation of CFTR and innate immune responses

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Cystic fibrosis (CF) is the most common genetically inherited disease in Caucasians (1 in 2500 newborns) and is caused by mutations of the cystic fibrosis transmembrane conductance regulator (CFTR). The most prevalent CFTR mutation $\Delta F508$ (which constitutes 70% of all mutations) results in an incorrect targeting of the CFTR molecule to the membrane. It is a well accepted concept that mucosal immune responses are dys-regulated in cystic fibrosis through a cycle of infectious (*Pseudomonas aeruginosa*/*P.a*) being an important pathogen in that disease)) and inflammatory episodes, and that there is an imbalance between proteases and antiproteases in that pathology, with a notable over-production of neutrophil elastase (NE), even in young CF infants (1).

This enzyme has been shown to degrade a number of innate immune receptors (eg complement receptors, CD2, CD4 and CD8 on lymphocytes (2-4), the 75-kDa form of the TNF-alpha receptor (5) on neutrophils), or receptors of apoptotic cells (6-7) as well as co-stimulatory receptors on dendritic cells (8). In addition, we have shown recently that NE has a deleterious effect on CFTR structure and CFTR channel function in epithelial cells *in vitro* and *in vivo* in a *P.a* lung infection model (9). Interestingly, we showed that NE inactivate CFTR through an intracellular calpain activation pathway. Importantly, experiments with NE^{-/-} mice indicated that other factors than NE may also be important in inducing the degradation of CFTR in our *P.a* infection model.

In that context, we have recently focused our studies on LasB, a member of the type II secretion system (T2SS) of *P.a*. It is a 33 kDa secreted zinc metallo-exoprotease encoded by the *lasB* gene. It has been shown to degrade several components of the extra-cellular matrix, and to breach the endothelial and epithelial cell barriers by attacking intercellular tight junctions (10-11). However, relatively little is known on its activity on innate immune receptors and protective mediators in the context of cystic fibrosis.

We will discuss here the effect of *P.a* in general, and LasB in particular, on CFTR structure and function, and their effect on the host lung epithelial innate immune responses.

Bibliography

- 1) Pillarisetti et al. Am J Respir Crit Care Med. 2011;184:75-81
- 2) Doring G et al. J Immunol. 1995;154:4842-50
- 3) Tosi MF et al. J Clin Invest. 1992;90:462-70
- 4) Remold-O'Donnell E et al. Blood. 1995;86:2395-402
- 5) Porteu F et al. J Biol Chem. 1991 ;266:18846-53
- 6) Vandivier RW et al. J Clin Invest. 2002;109:661-70
- 7) Henriksen PA et al. J Immunol. 2004;172:4535-44
- 8) Roghanian A et al. Am J Respir Crit Care Med. 2006;174:1189-98
- 9) Le Gars M, et al. Am J Respir Crit Care Med. 2013;187:170-9
- 10) Azghani AO Am J Respir Cell Mol Biol. 1996;15:132-40
- 11) de Bentzmann S et al. Lab Invest. 2000;80:209-19

S7.3 Proteases in the Cystic Fibrosis Lung – elastase and its ‘friends’

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Cystic Fibrosis (CF) lung disease accounts for the majority of the mortality associated with this genetic disorder. Although infection is thought to be a primary driver of the lung damage associated with CF lung disease, the ensuing neutrophilic response is thought to contribute significantly to the lung damage and excessive inflammation which is primary feature of this disease. It has been known for some time that neutrophil elastase (NE), a neutrophil serine protease, plays a significant role in promoting inflammation and mucus hypersecretion as well as inactivating important host defense proteins produced by the airway. We have recently engineered novel protease inhibitors that are resistant to NE-mediated inactivation. These new molecules, SLPI-AG and elafin-GG, demonstrate greater anti-inflammatory activities compared to their parent molecules in in vivo and ex vivo models of disease. We will also describe a role for the cysteinyl protease, cathepsin S (CatS), in the CF lung and discuss how this protease may contribute to lung inflammation.

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S7.4 Azithromycin effect on lung inflammation induced by *Pseudomonas aeruginosa* released proteases as shown by in vivo imaging in IL-8 transiently transgenized mice

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Azithromycin (AZM) is utilized in the treatment of chronic inflammatory diseases like diffuse panbronchiolitis, cystic fibrosis (CF) and COPD also for evidences of effects in contrasting lung inflammation, although the mechanisms are still under investigation. Recently, a mouse model has been developed transiently expressing the luciferase reporter gene under the control of an interleukin 8 (IL-8) bovine promoter (bIL-8 luc).

In the present study, the transgenic mouse has been used to in vivo observe IL-8 mediated lung inflammation induced by *Pseudomonas aeruginosa* secreted virulence factors, specifically metalloproteases (MP) and to test the possible anti-inflammatory effect of AZM. Culture supernatants (SN) from two *P. aeruginosa* strains (VR1; VR2), isolated from the sputum of sporadically infected CF patients, were obtained after growth in the absence or presence of a sub-MIC dose of AZM (\pm AZM). SNs were analyzed for the presence of MPs by using smart fluorescent probes that can be activated by proteases. VR1-AZM showed metalloprotease, pan-cathepsin and elastase activities while VR1+AZM lost them. VR2 \pm AZM SNs showed no protease activity. In vivo monitoring of lung inflammation after stimulation with *P. aeruginosa*

SNs has been conducted by imaging of bIL-8 luc transgenic mice. The pro-inflammatory activity of VR1-AZM was clearly visible 24 hours after the instillation while a significant decrease of this response was observed in presence of VR1+AZM supernatant. On the contrary, VR2-AZM did not induce any measurable inflammation in the mouse lung. BALF was recovered from IL-8 transgenic mice in order to evaluate the effect of VR1 and VR2 \pm AZM SN on inflammatory cell recruitment. VR1-AZM SN, containing MPs, stimulated recruitment of total cells and neutrophils while it was reduced after VR1+AZM challenge. Moreover, a selected panel cytokines resulted up-regulated after VR1-AZM instillation while their expression was lower in presence of VR1+AZM: IL-1 beta, IL-17, IL-12(p70), RANTES, KC. The VR2-AZM SN had an effect 4-fold lower as concern inflammatory cells recruitment.

These data suggest that bacterial proteases might be a target of AZM. The in vivo model here used for monitoring bacteria-induced inflammation might be applied to study pathogenesis of diseases such as cystic fibrosis and to evaluate the possible therapeutic effect of known and new molecules with a presumptive anti-inflammatory action.

This study was supported by Lega Italiana Fibrosi Cistica Associazione Veneta Onlus and Italian Cystic Fibrosis Research Foundation (FFC grant#18/2013).

S7.5 Neutrophil elastase-cleaved SPLUNC1 disrupts *Burkholderia cepacia* biofilms better than intact SPLUNC1Jean Tyrrell, Saira Ahmad, William Walton, Matthew Redinbo, Robert Tarran

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Cystic fibrosis is genetic multi-organ disease caused by absent or dysfunctional CFTR mediated Cl⁻ secretion which in the lung, leads to mucus obstruction and chronic infection/inflammation. Short Palate Lung and Nasal Epithelial Clone 1 (SPLUNC1) is an innate defense protein that is secreted by the upper airways. The *Burkholderia cepacia* complex (BCC) is a ubiquitous family of environmental microbes; some of which, for example, rot onions. Whilst generally harmless to humans, they are extremely pathogenic to CF patients and acquisition of BCC is associated with a significant increase in mortality. Whilst SPLUNC1 is known to have anti-microbial functions, its effects on the BCC are not known. Chronic neutrophilia is a hallmark of CF lung disease and SPLUNC1 function has been shown to be impaired following SPLUNC1 degradation with neutrophil elastase. We therefore tested the hypotheses that (i) SPLUNC1 was able to impair BCC biofilm formation and (ii) that neutrophil elastase impaired SPLUNC1's anti-biofilm actions. BCC clinical isolates from CF patients (J2315 and AU1054) were cultured as biofilms for 24 h under standard conditions. SPLUNC1 was then added over the range 0.1 – 100 µg/ml and biofilm growth measured. For J2315, ≥10 µg/ml SPLUNC1 disrupted biofilm formation. For AU1054, ≥1 µg/ml SPLUNC1 disrupted biofilm formation. We have recently elucidated the crystal structure of SPLUNC1. We therefore tested novel mutants based on this structure. D44 SPLUNC1, which lacks the S18 region, Δalpha4 and Δalpha6 which lack novel alpha helices all disrupted J2315 biofilm to a similar extent as WT SPLUNC1 (all n=3; p>0.05). In contrast, S18 peptide had no effect on J2315 biofilm growth (n=3; p>0.05). To cleave SPLUNC1, we exposed 1 µM SPLUNC1 to 100 nM NE for 1 h, after which time, we halted NE activity by adding the NE inhibitor ONO5046. Degradation of SPLUNC1 was confirmed by SDS-PAGE. NE ± ONO5046 had no effect on biofilm growth. Surprisingly however, the effect of SPLUNC1 to disrupt biofilm formation was significantly potentiated by NE pretreatment (n=3; p< 0.001). Whilst SPLUNC1's mechanism of action is not known, it appears that NE enhances SPLUNC1's ability to disrupt biofilms, suggesting that different components of the lung's innate immune system may work synergistically. Whilst the impact of CF on SPLUNC1-BCC biofilm interactions is not currently known, or data suggest that understanding this interaction may have important implications for CF lung disease.

We thank Dr. J LiPuma and the CFF for supplying the BCC isolates.

Funded by the NIH and the CF Trust.

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S7.6 Interactions of cytokeratin 8 with misfolded Phe508del-CFTR and Z-alpha-1 antitrypsin – role in protein trafficking and/or degradation

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Introduction and objective: We have reported that cytokeratin 8 (K8) affects the trafficking of misfolded Phe508del-CFTR from the endoplasmic reticulum (ER) to the plasma membrane by interacting directly with Phe508del-NBD1, and that disruption of this interaction leads to correction of functional Phe508del-CFTR (Colas et al., HMG, 2012, 21:623-34; Odolczyk et al., EMBO Mol Med, 2013, 5:1484-501). We hypothesize now that K8 interacts with other abnormally folded proteins, preventing their correct trafficking and secretion, and contributing to pathogenesis. We test this hypothesis on misfolded alpha-1 antitrypsin (A1AT), the Z mutant of A1AT (E342K). A1AT is an inhibitor of inflammatory proteases that protects the lungs from degradation. Misfolded but functional Z-A1AT mutant is degraded either by proteasome or by autophagy resulting in reduced secretion, what leads ultimately to lung and liver diseases. The aim of the study is to verify if A1AT/Z-A1AT form a complex with K8 by physical interaction and if this complex regulates A1AT secretion.

Methods: HeLa and CFBE (cystic fibrosis bronchial epithelial) cell lines, transiently transfected with cDNAs of A1ATs (WT or Z mutant) were used 48 h after transfection. The potential interaction between K8 and A1AT was verified by co-immunoprecipitation, and proximity ligation assay (PLA). Secretion of A1AT under different experimental conditions was tested by WB of secreted proteins. The effects of K8 concentration were investigated after silencing K8 expression by transduction of HeLa cells with shRNA. Direct interactions between K8 and A1AT variants were tested by surface plasmon resonance (SPR) on recombinant K8 and purified A1AT from human serum of healthy and ZZ-homozygous patients.

Results: Secretion of Z-A1AT was lower than of WT-A1AT 48h after transfection. WT- and Z-A1AT co-immunoprecipitated with K8 from both HeLa and CFBE cell extracts. PLA experiments showed that Z-A1AT localizes in a close proximity to K8 filaments (< 40 nm) in HeLa and CFBE cells. Number of potential interactions with K8 was higher for Z-A1AT than for WT-A1AT. Biophysical analysis by SPR confirmed that K8 physically interacts with Z-A1AT. K8 filaments network was modified in cells overexpressing Z-A1AT, as compared to WT-A1AT-expressing cells. Reduction of K8 expression in HeLa cells increased the secretion of Z-A1AT.

Conclusions: K8 physically interacts with Z-A1AT. Disruption of this interaction partially restores secretion of Z-A1AT.

We hypothesize that abnormal folding of the Z mutant potentially create cavities which stimulate direct interaction with K8. K8-A1AT complex represents a target for pharmacotherapy of A1AT deficiency. Altogether, K8 is an important regulator of transmembrane (CFTR) and soluble (A1AT) proteins maturation and trafficking in cells.

28 March — 16:15–18:00

Symposium 8 - CFTR trafficking

S8.1 The role of the heterotrimeric $G\alpha_{12}$ chaperone-like system in aberrant trafficking of CFTR channel.

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Cystic fibrosis (CF) is a genetic disease caused by mutations in the CF transmembrane regulator (CFTR) gene which encodes an epithelial chloride channel. The most frequent mutation, the deletion of phenylalanine at position 508, F508del, destabilizes the CFTR protein, thus causing a trafficking defect and degradation of the mutant CFTR by the ER-associated proteasomal degradation (ERAD) system. The trafficking defect can be overcome with drug-like molecules called correctors that are able to deliver the partially functional F508del-CFTR to the plasma membrane, but incapable to stabilize the rescued protein. The regulation of trafficking and degradation of F508del-CFTR is a complex process which involves a number of proteins including chaperones and adaptors. Thus manipulation of the chaperones might help to increase folding and trafficking efficiency of the mutant CFTR, allowing partial restoration of its function and membrane stability.

Interestingly, our recent investigation on the role of chaperones in ER-retention and degradation of F508del-CFTR, revealed the importance of a heterotrimeric G-proteins, the $G\alpha_{12}$, in mutant CFTR degradation and trafficking in relationship to calnexin (CNX) and HSP90 chaperones.

$G\alpha_{12}$ was predominantly localized in ER compartment and was found to interact with HSP90 and CNX. $G\alpha_{12}$ was shown to control HSP90 and CNX stability and indirectly control F508del-CFTR degradation. We showed that $G\alpha_{12}$, by interacting with HSP90 and CNX, controls the binding of these chaperones with F508del-CFTR in a GTP-dependent manner.

Based on these results, we propose that $G\alpha_{12}$ is a chaperone-like molecule that controls the association of F508del-CFTR with HSP90 and CNX proteins at the ER level. This model provides an upstream control of the ER-trafficking of CFTR protein.

S8.2 CFTR regulation by VIP: molecular mechanism and therapeutic potential

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VIP (Vasoactive Intestinal Peptide) is a 28-amino acid neuropeptide released from intrinsic neurons which controls multiple functions in exocrine tissues including inflammation, relaxation of airway and vascular smooth muscles and CFTR-dependent secretions contributing to mucus hydration and local innate defense of the lung. Early studies showed a drastic reduction of VIP-secreting nerve fibers in CF tissues, indicating a potential role for a lack of VIP secretion in CF development and progression. At the molecular level, in human airway epithelial cells, we found that VIP stimulates CFTR-dependent chloride secretion by 2 mechanisms: 1) an acute stimulation of CFTR activity by direct phosphorylation; and 2) a chronic stimulation which increases the density of active CFTR channels at the apical membrane of cells by reducing its endocytosis and increasing membrane insertion and stability at the cell surface. VIP activates the VPAC1 / PKC ϵ signaling cascade that phosphorylate the PDZ domain containing proteins Erzin/Radixin/Moesin (ERM), inducing the formation of a stable complex between CFTR, P-ERM and NHERF1 at the cell surface. Prolonged VIP stimulation also increases CFTR membrane insertion in a PKA-dependent manner via the dissociation of CFTR from CAL.

In vivo, features of lung and intestinal disease of VIP-KO mice resemble those reported in CF mice. Moreover, the absence of VIP induces the intracellular retention of CFTR and subsequent loss of chloride current, creating a CF-like condition. Intra-peritoneal injections of VIP corrected tissue abnormalities and CFTR dysfunction in VIP-KO mice.

Rescuing highly functional F508del-CFTR in CF epithelial cells by correcting its misfolding to promote membrane targeting while increasing surface stability is the major goal of current therapeutic strategies using corrector molecules.

We have tested the F508del-CFTR corrector potential of VIP and VIP-ELP biopolymer fusion molecules which are highly resistant to peptidases that rapidly degrade native VIP. In JME/CF15 human nasal epithelial cells derived from a F508del-homozygous CF patient, prolonged stimulation with VIP or VIP-ELP for 2-48 hrs at physiological temperature corrected F508del-CFTR trafficking and function in a dose-dependent manner (0.03 - 2.5 μ M) with EC₅₀ and plateau of activation in the range of 100nM and 1 μ M respectively.

Taken together, our results unveiled the important physiological role of VIP in the regulation of CFTR-dependent secretions in exocrine tissues and support the use of VIP derivatives for the treatment of cystic fibrosis at the molecular level for the vast majority of CF patients with the F508del mutation.

Supported by: CFC, NSERC, PhaseBio Pharmaceuticals Inc.

S8.3 Identification of novel HSP70 dependent mechanisms for degradation of rare CFTR mutants.

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Cystic Fibrosis is caused by a large number of mutations in CFTR that lead to aberrant folding and/or function. Progress has been made towards suppression of folding and functional defects in CFTR caused by the most common disease-related mutation, $\Delta F508$ [1, 2]. However, a large percentage of CF patients harbour mutant alleles other than $\Delta F508$ that cause misfolding and loss of cell surface expression [3, 4]. Missense mutations in membrane proteins lead to arrest of folding and accumulation of misfolded biogenic intermediates in different conformational states [5]. We are therefore investigating mechanisms by which rare CFTR disease alleles cause misfolding and are identifying CFTR mutants that exhibit correctable and non-correctable functional defects [6]. To define mutants that have correctable folding defects we've been investigating how misfolded mutants of CFTR (Class II) are recognized by Hsp70 and ERQC machinery. Mutants such as $\Delta F508$ accumulate in a misfolded state that can be brought back on pathway with folding correctors. Other mutants accumulate in a non-correctable state and are rapidly degraded by ERAD. We now report on the identification of a third type of class II mutants that accumulate in an ERAD-resistant conformation and are degraded by ERQC autophagy. HSP70 is required for folding and degradation of CFTR [7] and data will be presented that describes how HSP70 partitions different misfolded conformers between different degradation fates. Based on these data, we are starting to sub-divide class II mutants and grouping them into classes that are correctable and non-correctable with different type of tool compounds. This information is being translated to design corrector combinations for use in restoring function of rare CFTR mutants with unique folding defects.

References:

1. Ren, H.Y., et al., VX-809 corrects folding defects in cystic fibrosis transmembrane conductance regulator protein through action on membrane-spanning domain 1. *Mol Biol Cell*, 2013. 24(19): p. 3016-24.
2. Cyr, D.M., Arrest of CFTR $\Delta F508$ folding. *Nat Struct Mol Biol*, 2005. 12(1): p. 2-3.
3. Sosnay, P.R., et al., Defining the disease liability of variants in the cystic fibrosis transmembrane conductance regulator gene. *Nat Genet*, 2013. 45(10): p. 1160-7.
4. Van Goor, F., et al., Effect of ivacaftor on CFTR forms with missense mutations associated with defects in protein processing or function. *J Cyst Fibros*, 2013. 13(1): p. 29-36.
5. Houck, S.A., et al., Quality control autophagy degrades soluble ERAD-resistant conformers of the misfolded membrane protein GnRHR. *Mol Cell*, 2014. 54(1): p. 166-79.
6. Grove, D.E., et al., Mechanisms for rescue of correctable folding defects in CFTR $\Delta F508$. *Mol Biol Cell*, 2009. 20(18): p. 4059-69.
7. Younger, J.M., et al., Sequential quality-control checkpoints triage misfolded cystic fibrosis transmembrane conductance regulator. *Cell*, 2006. 126(3): p. 571-82.

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S8.4 Inhibition of Hsp90 ATPase activity leads to correct F508del-CFTR defective trafficking in cystic fibrosis cells

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The molecular chaperone heat shock protein 90 (Hsp90) plays an essential role in the maturation and functional activation of its client proteins. Further studies described Hsp90 inhibition as an effective strategy for cancer treatment (Chen *et al.*, 2014; Lindquist *et al.*, 2010). Previous work also demonstrated the efficiency of Hsp90 and Hsp70 pharmacological inhibition, by geldanamycin and 4-PBA respectively, in the correction of the F508del-CFTR defective trafficking (Fuller *et al.*, 2010; Singh *et al.*, 2008). In this work, we highlight the inhibitory effect of IsoLAB, a corrector of the F508del-CFTR (Best *et al.*, 2010), on Hsp90 ATPase activity and propose the inhibition of Hsp90 ATPase as a new pathway to correct F508del-CFTR defective trafficking.

Firstly, a series of competition experiments with several inhibitors of the ER quality machinery using the iodide efflux technique revealed the heat shock proteins (Hsp) as potential target of IsoLAB. We observed that thapsigargin, tunicamycin and miglustat, known to alter the F508del-CFTR/calnexin interaction, potentiate the F508del-CFTR rescue induced by IsoLAB. We also found that a treatment with MG132, a proteasome inhibitor, potentiate the IsoLAB effect. On the contrary, we did not observed a potentiation of IsoLAB-induced correction when cells were incubated at low temperature, a procedure known to rescue F508del-CFTR proteins via the inhibition of Hsp. Moreover, while 4-PBA, an Hsp70 inhibitor, slightly potentiated the effect of IsoLAB, no potentiation was observed when IsoLAB was associated in co-treatment with geldanamycin, an Hsp90 inhibitor. Thus, we used a Duolink "Proximity ligation Assay" to detect and quantify Hsps/CFTR interaction. We observed that IsoLAB decreased by 48% Hsp70/F508del-CFTR interaction and by 74% Hsp90/F508del-CFTR interaction resulting in the restoration of a functional F508del-CFTR to the plasma membrane. To determine whether IsoLAB inhibits these interactions, we measured the ATPase activity of Hsp90 and Hsp70. Interestingly; our results show an inhibition by 40% of the Hsp90 ATPase activity and no inhibition of the Hsp70 ATPase activity in presence of 100µM of IsoLAB. Therefore, we propose a new pathway to correct F508del-CFTR via the inhibition of Hsp90 proteins. Using iodide efflux, we observed that transfected cells with Hsp90 siRNA, decreased the Hsp90/CFTR interaction and corrected the abnormal trafficking of F508del-CFTR. We also demonstrated that three well known inhibitors of Hsp90, geldanamycin, 17-AAG and radicicol restore F508del-CFTR channel activity. However despite these molecules are potent inhibitors of Hsp90, they present a high cellular toxicity in a long-term treatment. On contrary, IsoLAB inhibits more weakly Hsp90 and could be a good alternative to restore F508del-CFTR defective trafficking without cellular toxicity.

To conclude, we described the inhibition of Hsp90 ATPase activity as a new pathway to correct F508del-CFTR defective trafficking and propose IsoLAB as a new Hsp90 inhibitor.

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S8.5 Sustained increases in cytosolic Ca²⁺ induce CFTR internalisationWaseema Patel^{1,2}, Abigail Marklew^{1,2}, Robert Tarran², Michael Gray¹

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Chronic obstructive pulmonary disease (COPD), the third leading cause of death worldwide, is characterised by airflow obstruction and is primarily caused by smoking (1). Another obstructive pulmonary disease, cystic fibrosis (CF), has orphan disease status and affects approximately 80,000 people worldwide. However, patients with either COPD or CF present with similar problems, including impaired mucus clearance from the airways due to production of thick, viscous mucus, brought about by defective ion homeostasis. In particular, CF transmembrane conductance regulator (CFTR) activity is reduced in both diseases. Recently, we have shown tobacco smoke exposure causes an increase in intracellular Ca²⁺ ([Ca²⁺]_i) leading to internalisation of CFTR, in both airway and non-airway cells, through an unknown mechanism (2, 3). We set out to investigate in more detail, the dynamics and molecular mechanism underlying the effect of an increase in [Ca²⁺]_i on CFTR channel activity using the fast whole cell configuration of the patch clamp technique, as well as real-time measurements of [Ca²⁺]_i by fluorescence microscopy. Thapsigargin (TG) was used to stimulate an increase in [Ca²⁺]_i in HEK 293T cells transiently transfected with CFTR. Cells were initially stimulated with forskolin to activate CFTR, and then exposed to TG for 10 mins to increase [Ca²⁺]_i. Current was monitored for a further 10 min wash out period in the presence of forskolin alone. Finally, cells were exposed to CFTR_{inh}-172 to test whether there was any residual CFTR-dependent current. Exposure to TG caused a time-dependent decrease in forskolin-activated CFTR conductance within 10 mins of exposure, with a ~60% reduction in conductance after 20 mins (initial conductance 2.3 ± 0.5 nS/pF; TG inhibited conductance 1.0 ± 0.2 nS/pF, n=10, p< 0.05). Addition of CFTR_{inh}-172 further inhibited the conductance to 0.3 ± 0.06 nS/pF (n=10, p< 0.05). The temporal effect of TG on CFTR was mirrored by changes in [Ca²⁺]_i; furthermore, the TG-induced reduction in CFTR channel activity was eliminated when experiments were repeated in the presence of a high concentration of EGTA in the patch pipette solution (initial conductance 3.0 ± 2.0 nS/pF; conductance post TG, 3.4 ± 2.0 nS/pF), showing that the rise in [Ca²⁺]_i was temporally related to the loss of CFTR conductance. Our lab has previously shown that tobacco smoke-induced Ca²⁺ release causes internalisation of CFTR into intracellular aggregates (3). We found that pre-incubation of cells with the endocytic inhibitor, Dynasore, blunted any decrease in CFTR activity by thapsigargin (initial conductance 3.0 ± 0.9 nS/pF; TG inhibited conductance, 2.5 ± 0.9 nS/pF). Addition of CFTR_{inh}-172 inhibited the conductance to 0.5 ± 0.3 nS/pF (n=7, p< 0.05). Together, these results show that a sustained increase in [Ca²⁺]_i decreased CFTR activity through internalisation of the channel via a dynamin-dependent endocytic route. Additional Ca²⁺ agonists and tobacco smoke components will be tested to further understand the physiological relevance and pathophysiological mechanism by which tobacco smoke-induced Ca²⁺ release affects CFTR activity.

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1. WHO (<http://www.who.int/mediacentre/factsheets/fs310/en/>); acc:06/08/2014
2. Clunes LA *et al.* 2012 *FASEB J* 26(2);533-45
3. Rasmussen JE *et al.* 2014 *JBC* 289(11);7671-81

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S8.6 Trafficking in CFTR therapies

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Cystic fibrosis (CF) is caused by loss of function mutations affecting CFTR, an ion channel that regulates ion and fluid homeostasis in epithelial tissues. The most common mutation is F508del-CFTR, which fails to fold, exhibits low channel activity, and is rapidly degraded. Existing CFTR modulators facilitate F508del folding and potentiate its ion-channel activity. However, none of them specifically target its post-maturational trafficking, and even in combination, they provide limited benefit (3% FEV₁) for F508del homozygotes and no benefit for heterozygotes. The Disabled-2 (Dab2) CLASP endocytic adapter protein and the CFTR-Associated Ligand (CAL) PDZ protein contribute to the endocytic uptake of F508del from the apical membrane of epithelial cells, limiting its half life and decreasing the net number of functional channels. Inhibitors of Dab2 and CAL are thus expected to extend the half-life of F508del and to increase net channel numbers at the membrane. Here, we present novel peptide and peptidomimetic inhibitors of CAL, designed to facilitate intracellular delivery. Using a mass-spectrometry-based pull-down assay, we demonstrate unprecedented selectivity for the CAL PDZ domain. We also have engineered a nanomolar peptide reporter for the Dab2 DH domain, and deployed it in a pilot HTS to identify small-molecule inhibitors. CAL and Dab2 inhibitors specifically enhance net ion-channel activity in airway epithelial cells. Furthermore, we show that such inhibitors not only increase channel activity on their own, but also can act additively or synergistically with existing folding correctors and gating potentiators. They thus offer the prospect of improved therapy for patients carrying the most common disease-associated CFTR mutation.

28 March — 18:15–19:15
Closing Keynote Lecture

Systems Approaches to Find Cystic Fibrosis Pathways and Therapies

Margarida D Amaral

Cystic Fibrosis (CF) is the most common genetic disease in Caucasians, caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR), a chloride channel expressed at the plasma membrane of epithelial cells which also regulates ENaC, the major epithelial Na⁺ channel. Defective CFTR thus originates impaired epithelial sodium and chloride transport which severely impacts on dehydrated epithelia in several organs, most notably the airways.

The F508del is the most common CF-causing mutation, occurring in ~85% of CF patients that leads to a misfolded protein which is retained in the endoplasmic reticulum (ER) and targeted for degradation by the ER quality control (ERQC), thus not reaching the cell surface.

Our goal is to approach CF by systems biology using functional genomics. We thus developed high-throughput microscopy screens to identify novel ENaC regulators and novel factors affecting the traffic of normal CFTR and those rescuing mutant F508del-CFTR to the cell surface. The latter constitute potential drug targets for CF.

Overall, our results point to a complex involvement of several cellular functions in the regulation of the CF physiopathology, leading to the identification of potential drug targets whose relevance in the context of basic cell and molecular biology and CF will be discussed.

References

[1] Botelho HM, Uliyakina I, Awatade NT, Proença MC, Tischer C, Sirianant L, Kunzelmann K, Pepperkok P, Amaral MD (2015) Protein Traffic Disorders: an Effective High-Throughput Fluorescence Microscopy Pipeline for Drug Discovery. *Sci Rep*. In Press.

[2] Alმაça J, Faria D, Sousa M, Uliyakina I, Conrad C, Sirianant L, Clarke LA, Martins JP, Santos M, Hériché JK, Huber W, Schreiber R, Pepperkok R, Kunzelmann K, **Amaral MD** (2013) High-content siRNA screen reveals global ENaC regulators and potential cystic fibrosis therapy targets. *Cell* **154**, 1390-1400. [PMID: 24034256].

[3] Farinha CM, King-Underwood J, Sousa M, Correia AR, Henriques MJ, Roxo-Rosa M, Da Paula AC, Williams J, Hirst S, Gomes CM, **Amaral MD** (2013) Revertants, Low Temperature, and Correctors Reveal the Mechanism of F508del-CFTR Rescue by VX-809 and Suggest Multiple Agents for Full Correction. *Chem Biol* **20**, 943-55. [PMID: 23890012].

[4] Alმაça J, Dahimène S, Appel N, Conrad C, Kunzelmann K, Pepperkok R, **Amaral MD** (2011) Functional genomics assays to study CFTR traffic and ENaC function. In: Cystic Fibrosis Protocols and Diagnosis. *Methods Mol Biol* **742**, 249-64. [PMID: 21547737].

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Molecular dynamics simulations on the wild-type and F508del CFTR 3D models

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In the absence of experimental 3D structures at atomic resolution for the entire CFTR protein, we and others have built homology models using as templates the experimental 3D structures of ABC exporters. These models provided significant insights into the structural and functional characteristics of CFTR, and were enriched by several molecular dynamics (MD) simulations, which now offer a description of the possible architecture of the anion channel in both the open and closed forms. Moreover, our recent MD simulation performed on wild-type CFTR suggests that the region in the first nucleotide-binding domain (NBD1) where F508 resides undergoes significant flexibility that allows promoting the open conformation of the channel through its interaction with the fourth intracellular loop (ICL4) (1).

Here, we specifically analysed a model we made of CFTR carrying the F508 deletion. This deletion results in a temperature-sensitive folding defect that impairs protein maturation and chloride channel function (2). It is however known that second-site suppressor mutations partially reverse these effects (3). Especially at the level of the ICL4:NBD1 interface, the R1070W measurably promotes the trafficking of the F508del protein and partially rescues the F508del CFTR phenotype. In order to understand, at the molecular level, the mechanisms by which this mutation impacts intermolecular interactions, we thus carried out MD simulations on the CFTR F508del/R1070W double mutant. These suggest that the side chain of the R1070W promotes a local reorganization of the NBD1 region where the F508 deletion is located (AA 498 to 515), making it able to fold as observed in the wild-type protein (1), whereas the channel can achieve a full open conformation.

We also wish to study, through MD simulations, the effect of other second-site suppressor mutations as well as of the simultaneous genetic stabilization of NBD1 energetics and NBD1-ICL4 interface, leading to robust and synergistic rescue of CFTR F508del folding and function (4,5). These theoretical studies should provide insightful information on the molecular mechanisms necessary for the F508del protein to be able to fold and function properly, which could be used to design efficient correctors.

1. Mornon JP*, Hoffmann B*, Jonic S, Lehn P., Callebaut I. 2015 Cell Mol Life Sci in press.
2. Thibodeau PH et al. 2010 J Biol Chem 285: 35825-35835.
3. Liu X, O'Donnell N, Landstrom A, Skach WR, Dawson DC 2012 Biochemistry 51:5113-5124.
4. Rabeh WM et al. 2012 Cell 148: 150-163.
5. Mendoza et al. 2012 Cell 148: 164-174.

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P2

Stability of CFTR and the effects of Cystic fibrosis-causing mutations in humans

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F508deletion and G551D in the cystic fibrosis transmembrane conductance regulator (CFTR) protein are the most frequent cystic fibrosis (CF)-causing missense mutations. Drugs VX-809 (a corrector) and VX-770 (a potentiator) are being used in clinical trials to target these mutations. Human wild-type (WT), F508del and G551D full-length CFTR were expressed in *S. cerevisiae* and purified in detergents n-dodecyl β -D-maltopyranoside (DDM) or lyso-phosphatidyl glycerol (LPG). For the first time, the thermal stability of the mutated proteins versus WT CFTR was studied in the absence and presence of the two drugs and nucleotides. We show that F508del CFTR is considerably less stable than WT and G551D versions of the protein. This instability can be partly rescued by the VX809 drug. The data has considerable significance for current clinical trials and our understanding of the molecular defect in CF. We have developed a medium throughput assay to detect small molecules that can correct CFTR stability. This leads to a simple model for the effects of the mutations at physiological temperatures and possible routes for therapy via small molecule correctors. The screen has also allowed us to identify conditions in which CFTR is relatively stable. This information is being fed into crystallization trials aimed at the determination of the high resolution structure of the full-length protein. We have been able to produce the protein in significant quantities and at concentrations well above 10 mg/ml.

P3

Towards an understanding of NBD1 and NBD2 heterodimerization

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Nucleotide binding by the first and second nucleotide binding domains (NBD1 and NBD2), heterodimerization of NBD1 and NBD2 and hydrolysis of the nucleotide at the heterodimer interface is thought to regulate opening and closing of the pore formed by the membrane spanning domains (MSDs) of CFTR. Based on CFTR NBD1 and other ABC transporter NBD crystal structures, the NBDs are presumed to heterodimerize in a "head to toe" manner with two nucleotide molecules sandwiched at the interface. The intrinsically disordered regulatory segments, including the regulatory insertion of NBD1 (RI) and R region, have been postulated to inhibit NBD heterodimerization, with inhibition being relieved by phosphorylation of these regions. The disordered C-terminus of CFTR may also participate in NBD heterodimerization. Neither biochemical nor structural studies have been successful in observing a direct interaction between the isolated NBD1 and NBD2 proteins, hindering our structural understanding of heterodimerization and the gating cycle. This is due in part to the extremely poor behavior of isolated NBD2. We use computational and biophysical tools to address NBD heterodimerization with the goal of understanding how this heterodimerization is controlled. Bioinformatic and structure simulation tools were used to suggest mutations that would improve the behavior of NBD2. As a result, we are now able to produce NBD2 with significantly improved behavior for biophysical characterization including fluorescence and NMR studies. We have previously presented data from NMR studies that provide evidence of NBD1 homodimerization, which we found to be impaired by the F508del mutation. However, preliminary NMR studies failed to provide concrete evidence of NBD1:NBD2 heterodimerization. To address this, we are using paramagnetic relaxation enhancement (PRE) to further probe heterodimerization. Soluble single cysteine mutants of NBD1 were generated to allow attachment of a single cysteine-linked spin label. Control experiments verified that these PRE experiments enable observation of NBD1 homodimers. We provide a progress update on PRE and fluorescence experiments designed to observe NBD heterodimerization. With these new reagents, we aim to probe the effects of nucleotide binding, F508del and other disease causing mutations, the C-terminus, the RI and the R region (both phosphorylated and non-phosphorylated) on NBD1:NBD2 dimerization. This work will also enable studies of small molecule modulators targeted at the NBD dimer interface. This work was funded by grants from the Cystic Fibrosis Foundation Therapeutics and Cystic Fibrosis Canada.

P4

Motions of CFTR membrane spanning helices related to channel gating

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The paucity of CFTR high-resolution 3D structural information has impeded elucidation of its mechanism of action and understanding of the impact of disease causing mutations. Homology models developed employing ABC exporters of known structure as templates have contributed somewhat towards realization of these objectives. However because of the very low sequence similarity between CFTR and the templates it is not expected that they very precisely mirror each other, especially in the membrane domains. Nevertheless, data from the influence of mutagenesis, labeling, and cross-linking experiments (Linsdell P. *Mol Membr Biol.* 2014, 31:1-16.) suggest that the overall arrangements of membrane spanning helices (TMs) is consistent with that predicted by the models. However, there is little information about the motions of these helices that may be involved in channel gating. To address this issue we have iteratively applied computational modeling tools including discrete molecular dynamics simulations to reconstruct robust structural models of CFTR by introducing cysteine cross-linking/single channel measurements as experimental constraints. The data reveal that the crosslinking of a key residue in TM6 (R352C) with one in TM12 (W1145C) locks the channel closed. Consistent with the previous finding that R352 forms salt bridge with D993 in TM9 (Cui G. et al. *J Biol Chem.* 2013, 288:20758-67), our data implicates that these residues could be reversibly cross-linked. However, the functional impact of this pair could not be evaluated as the channel closed upon cross-linking. Strikingly, spontaneous oxidative disulfide linkage between cysteines replacing residues near the extracellular ends of TMs within the same "wing" of the modeled ABC structures and not expected to primarily influence OWF-IWF transitions reversibly locked the channel closed (e.g., D112C/T1122C and A326C/V905C) as did pairs across the two "wings" that might be expected to have such an influence (eg. L333C/G1127C). From these integrated computational and experimental analyses, we conclude that channel gating transitions depend on motions between multiple membrane helices and fixation in a stable open state by a single inter-helical restriction may not be readily achieved.

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P6

EPAC1 activation by high levels of cAMP stabilizes CFTR at the membrane through interaction with NHERF-1

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Background: CFTR-mediated ion transport is stimulated by increase in subcortical cAMP levels through protein kinase A (PKA) activation thus triggering CFTR phosphorylation and channel opening. However, besides PKA, exchange proteins (EPACs) are also directly activated by cAMP by direct binding of this second messenger. EPACs are guanine nucleotide exchange factors (GEFs) for Rap family small GTPases, regulating cell-to-cell and cell-to-matrix adhesion, cytoskeleton rearrangements and cell polarization, processes which are dysregulated in CF. In response to cAMP, EPACs shift to the plasma membrane (PM) anchoring through ezrin-radixin-moesin (ERM) proteins, known to link CFTR to the actin cytoskeleton and to facilitate cAMP-driven CFTR activation by tethering PKA in their close proximity. This suggests that activation of both CFTR and EPACs might be spatially and temporally coincident, but little is known of the involvement of EPACs in CFTR biology.

Aim: We aimed to characterize the interaction between EPAC1 and CFTR and to elucidate the impact of EPAC1 on CFTR biogenesis, processing, trafficking and PM anchoring.

Methods: Western blot (WB), co-immunoprecipitation (co-IP), cell surface biotinylation, live cell imaging and FRET. Lung epithelial cell lines: A549 cells expressing wt- or F508del-mCherry-CFTR under a Tet-ON promoter, CFBEs stably expressing wt- or F508del-CFTR, Calu3 and HEK293T cells.

Results: Our results show that:

(i) EPAC1 activation with the cAMP analogue 007-AM (a membrane-permeable EPAC-specific agonist not activating PKA) leads to its translocation from cytosolic and perinuclear regions to the PM, in CFBE cells.

(ii) EPAC1 interacts with NHERF1 and this interaction is further promoted by EPAC activation.

(iii) EPAC1 co-localizes and is present in protein complexes involving wt- or F508del-CFTR and this interaction is mediated by NHERF1 but not ezrin.

(iv) 007-AM-activated EPAC leads to its increased association with CFTR and increased CFTR PM levels, without affecting CFTR steady-state processing efficiency (band C/band B ratio).

(v) Increase in endogenous cAMP levels, through forskolin-activated adenylate cyclase (AC), increases the membrane levels of CFTR.

(vi) EPAC1 knockdown (by siRNA or shRNA) leads to decreased of CFTR PM levels in different cell lines.

(vii) Treatment with 007-AM promotes cell adhesion when compared to the DMSO control in CFBE cells expressing wt-CFTR, but not in CFBE cells expressing F508del-CFTR.

Conclusion: Our results establish that EPAC1 associates to CFTR in membrane complexes with NHERF-1, suggesting an involvement of EPAC1 in the late stages of CFTR biogenesis, and supporting a role for EPAC1 in enhancing CFTR traffic to the PM, making it a novel potential therapeutic target for CF. These data indicate that cAMP signalling regulates CFTR through two distinct but complementary pathways – PKA and EPAC1.

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P7

Sustained increases in cytosolic Ca²⁺ induce CFTR internalisation

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Chronic obstructive pulmonary disease (COPD), the third leading cause of death worldwide, is characterised by airflow obstruction and is primarily caused by smoking (1). Another obstructive pulmonary disease, cystic fibrosis (CF), has orphan disease status and affects approximately 80,000 people worldwide. However, patients with either COPD or CF present with similar problems, including impaired mucus clearance from the airways due to production of thick, viscous mucus, brought about by defective ion homeostasis. In particular, CF transmembrane conductance regulator (CFTR) activity is reduced in both diseases. Recently, we have shown tobacco smoke exposure causes an increase in intracellular Ca²⁺ ([Ca²⁺]_i) leading to internalisation of CFTR, in both airway and non-airway cells, through an unknown mechanism (2, 3). We set out to investigate in more detail, the dynamics and molecular mechanism underlying the effect of an increase in [Ca²⁺]_i on CFTR channel activity using the fast whole cell configuration of the patch clamp technique, as well as real-time measurements of [Ca²⁺]_i by fluorescence microscopy. Thapsigargin (TG) was used to stimulate an increase in [Ca²⁺]_i in HEK 293T cells transiently transfected with CFTR. Cells were initially stimulated with forskolin to activate CFTR, and then exposed to TG for 10 mins to increase [Ca²⁺]_i. Current was monitored for a further 10 min wash out period in the presence of forskolin alone. Finally, cells were exposed to CFTR_{inh}-172 to test whether there was any residual CFTR-dependent current. Exposure to TG caused a time-dependent decrease in forskolin-activated CFTR conductance within 10 mins of exposure, with a ~60% reduction in conductance after 20 mins (initial conductance 2.3 ± 0.5 nS/pF; TG inhibited conductance 1.0 ± 0.2 nS/pF, n=10, p< 0.05). Addition of CFTR_{inh}-172 further inhibited the conductance to 0.3 ± 0.06 nS/pF (n=10, p< 0.05). The temporal effect of TG on CFTR was mirrored by changes in [Ca²⁺]_i; furthermore, the TG-induced reduction in CFTR channel activity was eliminated when experiments were repeated in the presence of a high concentration of EGTA in the patch pipette solution (initial conductance 3.0 ± 2.0 nS/pF; conductance post TG, 3.4 ± 2.0 nS/pF), showing that the rise in [Ca²⁺]_i was temporally related to the loss of CFTR conductance. Our lab has previously shown that tobacco smoke-induced Ca²⁺ release causes internalisation of CFTR into intracellular aggregates (3). We found that pre-incubation of cells with the endocytic inhibitor, Dynasore, blunted any decrease in CFTR activity by thapsigargin (initial conductance 3.0 ± 0.9 nS/pF; TG inhibited conductance, 2.5 ± 0.9 nS/pF). Addition of CFTR_{inh}-172 inhibited the conductance to 0.5 ± 0.3 nS/pF (n=7, p< 0.05). Together, these results show that a sustained increase in [Ca²⁺]_i decreased CFTR activity through internalisation of the channel via a dynamin-dependent endocytic route. Additional Ca²⁺ agonists and tobacco smoke components will be tested to further understand the physiological relevance and pathophysiological mechanism by which tobacco smoke-induced Ca²⁺ release affects CFTR activity.

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1. WHO (<http://www.who.int/mediacentre/factsheets/fs310/en/>); acc:06/08/2014
2. Clunes LA *et al.* 2012 *FASEB J* 26(2);533-45
3. Rasmussen JE *et al.* 2014 *JBC* 289(11);7671-81

P8

Exploring the mechanisms behind cigarette smoke-induced internalization of the cystic fibrosis transmembrane conductance regulator

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Chronic Obstructive Pulmonary Disease (COPD) is a leading health problem, with an estimated 80 million cases worldwide. Cigarette smoke (CS) exposure is the predominant cause of COPD but interestingly, patients with cystic fibrosis (CF), a disease caused by genetic mutation in the CF transmembrane conductance regulator (CFTR) gene, have similar pathological features to COPD such as mucus dehydration and a failure of mucociliary clearance. Recently our laboratory has proposed that these characteristics are in part caused by the rapid internalization and aggregation of CFTR following cigarette smoke (CS) exposure (1). However the molecular mechanisms underlying CS-induced CFTR internalization remain unexplored. To this end, we set out to characterize the mechanisms behind the CS-induced internalization of CFTR. In HEK293T cells transfected with GFP CFTR, air exposure (control) did not change the fluorescence intensity or subcellular localization of CFTR after incubation time intervals up to 60 min, whereas exposure to 10 puffs of freshly generated CS decreased CFTR membrane fluorescence intensity, with a half-life of 10.2 min ($n = 45 - 80$ cells) and a τ of 14.7, and intracellular CFTR appeared with similar kinetics. In a healthy state, CFTR undergoes clathrin-mediated endocytosis and recycling. Hypertonic sucrose has been shown to inhibit the formation of clathrin-coated pits (2). Thus, HEK293T cells transiently expressing GFP-CFTR were pre-treated with 0.45 M hypertonic sucrose, the cells were exposed to CS or air as described above and then incubated for 60 min and fixed in 4 % paraformaldehyde. Before treatment with sucrose, CS exposure caused intracellular GFP-CFTR fluorescence intensity to increase from 0.4 ± 0.03 to 1.0 ± 0.06 A.U. (mean \pm SEM, $n = 156 - 157$ cells), however, after sucrose-treatment, CS exposure only increased intracellular GFP-CFTR fluorescence intensity from 0.3 ± 0.04 to 0.5 ± 0.06 A.U. (mean \pm SEM, $n = 81 - 127$ cells) indicating that hypertonic sucrose inhibits CS-induced internalization. These data suggest that post CS, CFTR is internalized via clathrin-coated vesicles. Further to this, dynamin is a known GTPase responsible for pinching off clathrin-coated vesicles from the cell membrane (3). Pretreatment with Dynasore, a dynamin inhibitor, prevented CS-induced CFTR internalization confirming that dynamin is required for this internalization. We have also investigated the localization of CFTR post-CS induced internalization using HEK293T cells. Here, GFP-CFTR was co-expressed with Rab 5A, an early endosome marker. After a smoke exposure time course as above, CFTR colocalization with Rab 5A increased from $34.9 \% \pm 3.64$ to $58.1 \% \pm 4.5$ (mean \pm SEM, $n = 22$ cells) at 60 min, indicating that CFTR relocates to early endosomes after CS exposure. Our data suggest that CS-induced internalization is carried out through a clathrin and dynamin mediated pathway to early endosomes. Future experiments will investigate the later stages of internalization leading to aggregation of CFTR.

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¹Clunes, LA *et al.* (2012) *FASEB J.* 26(2):533-45.

² Heuser, JE *et al.* (1989) *J Cell Biol.* 108(2):389-400

³Warnock DE *et al.*(1996) *Bioessays.* 1996 18:885–893

P9

Anoctamins control epithelial Cl⁻ secretion by CFTR

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In cystic fibrosis, defective Cl⁻ secretion by mutant CFTR might be compensated by alternative chloride channels such as Ca²⁺ activated TMEM16 Cl⁻ channels. TMEM16A (anoctamin 1, ANO1) is expressed in epithelial tissues relevant to cystic fibrosis, like epithelial cells of airways, intestine and pancreas. We examined whether TMEM16A forms an independent secretory Cl⁻ channel or whether it operates in conjunction with CFTR, as previous work suggested. In mouse intestine lacking expression of TMEM16A, Ca²⁺ dependent Cl⁻ secretion was significantly reduced, although TMEM16A was found to be expressed in the basolateral membrane of enterocytes. These data indicated that i) CFTR might be the only apical exit pathway for mouse intestinal Cl⁻ secretion, similar to human intestine, ii) TMEM16A may facilitate Ca²⁺ signaling to activate basolateral Ca²⁺ dependent K⁺ channels to increase driving force for CFTR dependent Cl⁻ secretion, and iii) TMEM16A controlled Ca²⁺ signaling possibly activates apical CFTR by inhibition of Ca²⁺-dependent phosphatases and/or activation of PKC. We further examined in a recombinant cell system whether only TMEM16A or also other proteins of the TMEM16 family change intracellular Ca²⁺ signaling. Therefore we overexpressed ANO1, 4, 5, 6, 7, 8, 9 and 10 in HeLa cells and measured changes of intracellular calcium concentration close to the plasma membrane, using the plasma membrane targeted calcium sensitive GFP protein. We found that overexpression of ANO 1, 5, 6 and 10 enhanced intracellular Ca²⁺ signaling elicited by purinergic stimulation with 100 μM ATP. We further studied cellular localization of overexpressed ANO10 in HEK293 cells. Membrane biotinylation and costaining with ER tracker indicated that most of ANO10 was located in the endoplasmic reticulum (ER) rather in the plasma membrane. However, cellular localization was depending on cellular proliferation. We propose that ANO10 and possibly other anoctamins can act as a counter ion channel for calcium store release from the ER, if partially located in the ER. Taken together, anoctamins may control epithelial Cl⁻ transport either by acting as apical Cl⁻ exit channels or by facilitating CFTR dependent Cl⁻ secretion due to enhanced intracellular Ca²⁺ signaling.

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P10

Multielectrode biosensor system to study ion fluxes across the monolayer of epithelial cells

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Epithelia cover all wet surfaces of the body. Tightly bound epithelial cells form a barrier for water and ion transport in lungs, pancreatic ducts and sweat glands. There are different ion channels, transporters and pump molecules present in epithelial cell membranes. The defect in single anion channel CFTR is responsible for the most common fatal human genetic disorder – cystic fibrosis. The defect in the CFTR ion channel changes passive osmotic water transport across epithelium. The change in water transport leads to dense mucus formation prone to opportunistic bacterial infections. Multiple ions are transported across the epithelial cell monolayer i.e. sodium, potassium, chloride, hydrogen and bicarbonate. Measurement of all these ions as fast as possible is the key to understand the mechanism of cystic fibrosis. In our laboratory we built and successfully tested the extremely small volume multielectrode ion selective electrode biosensor system which can measure transport of all these ions but bicarbonate.

The 16HBE-14 σ cell line was used for experiments. It forms tight junctions and is characterized by high transepithelial electrical resistance (TER). Cells were seeded onto Costar Snapwell inserts (0.45 μm pore size, 1 cm^2 surface area) and grown in submerged culture medium until the TER of the cell monolayer was about 1000 Ω . To polarize the cells, the medium from the upper side of the insert was aspirated to maintain the air contact at the apical side of the monolayer. When the TER value decreased to about 400 Ω the monolayer was used in experiments.

First measurements made on our multielectrode biosensor equipped with two chloride, two sodium and single reference electrodes on each side of epithelial monolayer showed, that sodium is transported via paracellular route while chloride by transcellular one (defective in CF). Recent studies were performed on bisensor equipped with sodium, potassium, chloride, pH and reference electrode on each side of the polarized cell monolayer. Experiments show that ion transport across 16HBE-14 σ is very complex phenomenon. After medium change all ions were transported simultaneously through the cell layer. For example introduction of chloride gradient across the cell monolayer causes not only transport of chloride ions but also transport of potassium ions and pH change. Blocking of one particular ion channel species affects transport of all the ions across the cell layer.

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P11

Analysis of CFTR channel gating by hydrolysable ATP analogues with substitutions in the ribose sugar of ATP

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Cystic fibrosis (CF) is caused by dysfunction of the cystic fibrosis transmembrane conductance regulator (CFTR) an ATP-binding cassette (ABC) transporter, which functions as a Cl⁻ channel gated by intracellular ATP. To understand better ATP-dependent CFTR channel gating, we investigated channel gating by 2'-deoxy ATP (2'-dATP) and five other hydrolysable ATP analogues with substitutions in the ribose sugar of ATP selected because of their differing physical and chemical properties

(2'-O-methyl-ATP, 2'-F-ATP, 2'-Cl-ATP, 2'-Br-ATP and 2'-I-ATP). To study CFTR channel gating, we used excised inside-out membrane patches from C127 and BHK cells expressing wild-type (WT-) CFTR and low temperature-rescued F508del- and A561E-CFTR. With the exception of 2'-dATP and 2'-Cl-ATP, all ATP analogues reduced macroscopic WT-CFTR currents by 26 – 43% (n = 3 – 7). By contrast, 2'-Cl-ATP generated WT-CFTR currents similar in magnitude to ATP, whereas those of 2'-dATP were increased by ~50% (n = 3 – 5). Consistent with these data, 2'-O-methyl-ATP, 2'-F-ATP, 2'-Br-ATP and 2'-I-ATP all reduced open probability (P_o), 2'-Cl-ATP and ATP had equivalent P_o values, while that of 2'-dATP was markedly increased; no ATP analogue altered single-channel current amplitude. To understand better WT-CFTR channel gating, we performed an analysis of bursts. The mean burst duration (MBD) and interburst interval (IBI) of 2'-Cl-ATP (0.3 mM) (MBD, 143 ± 17 ms; IBI, 288 ± 139 ms; n = 3) did not differ from those of ATP (0.3 mM) (MBD, 119 ± 4 ms; IBI, 238 ± 91; n = 5; P > 0.05), while 2'-dATP (0.3 mM) increased the frequency and duration of channel openings (MBD, 178 ± 16 ms; IBI, 115 ± 15 ms; n = 5; P < 0.05). Analysis of concentration-response relationships suggested that 2'-dATP gates WT-CFTR with higher affinity and greater efficacy than ATP (K_d: ATP, 260 μM; 2'-dATP, 85 μM; P_{o(max)}: ATP, 0.55; 2'-dATP, 0.71; n = 3 – 14). Because 2'-dATP potentiated strongly WT-CFTR and rescued the gating mutant G551D-CFTR [1], we tested its effects on F508del- and A561E-CFTR, a CF mutation with a similar mechanism of dysfunction as F508del-CFTR [2]. Interestingly, 2'-dATP potentiated F508del-CFTR channel gating by increasing the frequency and duration of channel openings, but was without effect on A561E-CFTR channel gating (n ≥ 4). Nevertheless, 2'-dATP failed to influence the plasma membrane instability of F508del- and A561E-CFTR. At 37 °C, in the presence of ATP or 2'-dATP mutant channel activity declined rapidly and was lost completely after ~8 minutes (n ≥ 4). We conclude that altering the chemical group at the 2'-position of ATP changes CFTR gating, suggesting that this position plays a regulatory role. Our data further suggest that these ATP analogues might be valuable tools to investigate CFTR channel gating and inform the design of innovative CFTR potentiators for CF drug development.

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References:

[1] Cai Z et al. J Biol Chem. 2006; 281:1970-7.

[2] Wang Y et al. Br J Pharmacol. 2014; 171:4490-503.

P12

Safety and efficacy of short palate and lung nasal epithelial clone 1 (SPLUNC1)-derivatives for the treatment of CF lung disease

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In CF, the loss of CFTR function is associated with concomitant ENaC hyperactivity, which contributes to mucus dehydration in CF lungs. SPLUNC1 is secreted by airway epithelia and is a key regulator of ENaC. We found that the inhibition of ENaC by the SPLUNC1 protein is pH dependent: SPLUNC1 is effective at regulating ENaC and airway surface liquid (ASL) height in normal human bronchial epithelial cultures (HBECs) at physiological pH but does not inhibit ENaC at acid pH present in CF lungs. The lack of ENaC inhibition by SPLUNC1 in CF airways permits Na⁺ hyperabsorption and dehydration of ASL volume. To bypass the pH effect on SPLUNC1, we isolated an 18 residue peptide (S18) that is the functional ENaC inhibitory domain of SPLUNC1. S18 selectively binds to bENaC and inhibits Na⁺/ASL absorption in both normal and CF HBECs in a pH-independent manner. S18 is resistant to proteolytic degradation. In this study, we tested the hypothesis that S18 was capable of preventing lung disease in SCNN1B transgenic mice. To confirm that S18 was functional in murine tracheal epithelial cultures (MTECs), the change in ASL height in MTECs was measured ± S18 by confocal microscopy. At 4 h post-treatment, vehicle treated cultures showed an ASL height of $10.7 \pm 2.9 \mu\text{m}$ compared to $21.6 \pm 0.7 \mu\text{m}$ for the S18 group ($p < 0.05$). The addition of S18 also had a significant effect on MTECs isolated from SCNN1B mice. At 4 h post-treatment, vehicle treated SCNN1B MTECs showed an ASL height of $4.5 \pm 0.2 \mu\text{m}$ compared to $18.9 \pm 0.6 \mu\text{m}$ for the S18 group ($p < 0.05$). We then delivered S18 (10 mM at 1 μl per g body weight) to newborn SCNN1B pups twice daily for 14 days by intranasal instillation and AB-PAS staining on these mice showed significant differences in intraepithelial mucus volume density. The vehicle treated SCNN1B mice showed an intraepithelial mucus volume density of $6.9 \pm 1.8 \text{ nl/mm}^2$ compared to $0.9 \pm 0.3 \text{ nl/mm}^2$ for the S18 group ($p < 0.05$). The results for the S18 group were comparable to vehicle treated WT mice, which suggests that S18 may prevent goblet cell metaplasia and the clinical sequelae associated with CF lung disease in vivo. Since ENaC is also responsible for Na⁺ absorption in the nephron, a concern of any potential ENaC inhibitor is that it may have undesirable renal effects. To test the effects of S18 on renal homeostasis, we injected WT mice i.p. with isotonic saline containing S18, amiloride, or vehicle. Measuring urine volume, Na⁺, and K⁺ output over 8 h, we saw no difference between the S18 and the vehicle group. Amiloride treatment produced a significant increase in urine volume and Na⁺ output and a decrease in K⁺ output. Analysis of the peptide composition has allowed for the rational development of optimized compounds with sub-nM potency at increasing the ASL height and in a CFTR mutation independent manner. The physiological activity of these optimized derivatives will be discussed.

P13

Iminosugars acting as mannosidase inhibitors in cystic fibrosis: design of multivalent correctors and identification of new therapeutically targets

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In numerous loss-of-function genetic diseases as Cystic Fibrosis (CF), missense mutations lead to degradation of the mutated proteins by the ER conformation-based quality control (ERQC). One way of tagging a glycoprotein for degradation is through mannose trimming, making ER mannosidase a potential key player of the process. We screened a small chemical library of iminosugars on the activity of F508del-CFTR using our Bioscreen platform (iodides effluxes) and revealed a corrector effect of inhibitors of the ER α 1,2-mannosidases as kifunensine, swainsonine, deoxymannojirimycin (DMJ) and its n-butyl derivative nB-DMJ. In the present study, our objectives are to design, synthesize, screen and validate new iminosugars derived from the multivalency concept as probes and/or pharmacological chaperones to get insights and rescue of trafficking-deficient diseases, especially CF, and to explore the role of ER mannosidase and its partners on the ERQC.

First, we adapted a “click’chemistry” methodology recently reported (Compain, et al., 2013) which should facilitates synthesize of more efficient F508del-CFTR correctors based mainly on DMJ multivalents. From this screening performed on F508del-CFTR transfected Hela cells, we demonstrated that the trivalent (AJ47) and the tetravalent iminosugars present the best efficiency with an EC_{50} of 0.8 μ M, ~ 200-fold more efficient than the bivalent, the monovalent or than DMJ. We confirmed the corrector effect of the trivalent AJ47 on human polarized bronchial epithelial cells (CFBE) using the Ussing Chamber technique with a restoration of an I_{sc} current of 20 μ A.cm² in CFBE monolayers treated with AJ47. We also confirmed the corrector effect of AJ47 on human respiratory epithelial cells freshly isolated from CF patients using the fluorescent probe oxonol. Then, we performed competition experiments between AJ47 and inhibitors of ERQC. We observed a potentiation of the AJ47 effect in presence of MG132 a proteasome inhibitor, geldanamycin an HSP90 inhibitor or miglustat an iminosugar inhibitor of the ER glucosidases. On contrary, no potentiation was observed in presence of kifunensine or swainsonine. These results suggest a specific action of AJ47 on the ER mannosidase. We completed these results using the Duolink assay to determine the effect of AJ47 on the F508del-CFTR interactions with the main chaperones of the ERQC. We did not observe effect on the interaction with calnexin, HSP70 nor HSP90. On the contrary, we found a decrease of 35% of the interaction between F508del-CFTR and the lectin EDEM (ER Degradation-enhancing α mannosidase like protein) involved in the degradation pathway, without altering its expression level or the expression level of the other key proteins of quality control system.

To conclude, our results demonstrate for the first time that second generation iminosugars, inhibitors of ER α 1,2-mannosidases, correct the defective trafficking of F508del-CFTR with an EDEM-dependent mechanism of action. These compounds are water soluble, not toxic and more efficient than first generation iminosugars.

P14

Inhibition of Hsp90 ATPase activity leads to correct F508del-CFTR defective trafficking in cystic fibrosis cells

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The molecular chaperone heat shock protein 90 (Hsp90) plays an essential role in the maturation and functional activation of its client proteins. Further studies described Hsp90 inhibition as an effective strategy for cancer treatment (Chen *et al.*, 2014; Lindquist *et al.*, 2010). Previous work also demonstrated the efficiency of Hsp90 and Hsp70 pharmacological inhibition, by geldanamycin and 4-PBA respectively, in the correction of the F508del-CFTR defective trafficking (Fuller *et al.*, 2010; Singh *et al.*, 2008). In this work, we highlight the inhibitory effect of IsoLAB, a corrector of the F508del-CFTR (Best *et al.*, 2010), on Hsp90 ATPase activity and propose the inhibition of Hsp90 ATPase as a new pathway to correct F508del-CFTR defective trafficking.

Firstly, a series of competition experiments with several inhibitors of the ER quality machinery using the iodide efflux technique revealed the heat shock proteins (Hsp) as potential target of IsoLAB. We observed that thapsigargin, tunicamycin and miglustat, known to alter the F508del-CFTR/calnexin interaction, potentiate the F508del-CFTR rescue induced by IsoLAB. We also found that a treatment with MG132, a proteasome inhibitor, potentiate the IsoLAB effect. On the contrary, we did not observed a potentiation of IsoLAB-induced correction when cells were incubated at low temperature, a procedure known to rescue F508del-CFTR proteins via the inhibition of Hsp. Moreover, while 4-PBA, an Hsp70 inhibitor, slightly potentiated the effect of IsoLAB, no potentiation was observed when IsoLAB was associated in co-treatment with geldanamycin, an Hsp90 inhibitor. Thus, we used a Duolink "Proximity ligation Assay" to detect and quantify Hsps/CFTR interaction. We observed that IsoLAB decreased by 48% Hsp70/F508del-CFTR interaction and by 74% Hsp90/F508del-CFTR interaction resulting in the restoration of a functional F508del-CFTR to the plasma membrane. To determine whether IsoLAB inhibits these interactions, we measured the ATPase activity of Hsp90 and Hsp70. Interestingly; our results show an inhibition by 40% of the Hsp90 ATPase activity and no inhibition of the Hsp70 ATPase activity in presence of 100µM of IsoLAB. Therefore, we propose a new pathway to correct F508del-CFTR via the inhibition of Hsp90 proteins. Using iodide efflux, we observed that transfected cells with Hsp90 siRNA, decreased the Hsp90/CFTR interaction and corrected the abnormal trafficking of F508del-CFTR. We also demonstrated that three well known inhibitors of Hsp90, geldanamycin, 17-AAG and radicicol restore F508del-CFTR channel activity. However despite these molecules are potent inhibitors of Hsp90, they present a high cellular toxicity in a long-term treatment. On contrary, IsoLAB inhibits more weakly Hsp90 and could be a good alternative to restore F508del-CFTR defective trafficking without cellular toxicity.

To conclude, we described the inhibition of Hsp90 ATPase activity as a new pathway to correct F508del-CFTR defective trafficking and propose IsoLAB as a new Hsp90 inhibitor.

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P15

rAAV2/5 encoding truncated CFTR rescues CF phenotype in Δ F508 mouse model

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INTRODUCTION: To date, the majority of CF patients receive only symptomatic treatment. Gene therapy offers the potential to cure the disorder in a mutation-independent manner. Viral vector-mediated gene transfer exploits the natural and efficient properties of viruses to enter and persist in the host cell. Although clinical trials for CF gene therapy only resulted in limited success so far, recent advances in the gene therapy field for other diseases, such as the market authorization of a rAAV-based gene therapy vector for lipoprotein lipase deficiency which the EMA granted in 2012, encouraged us to re-explore gene therapy for CF. For our studies we use improved rAAV technology, first an rAAV vector with airway tropism (rAAV2/5) and second, an optimized CFTR expression cassette (1). Here, we evaluated the therapeutic potential of a rAAV2/5 encoding a truncated CFTR in a Δ F508 mouse model.

RESULTS: First, we assessed rAAV2/5 tissue tropism and stability of gene expression using reporter genes (Fluc and β -gal). A single administration of rAAV2/5 in adult mice resulted in relatively stable gene expression up to 15 months with only a 4-fold decrease in bioluminescence signal in lungs. Next, to overcome the size limit of rAAV vectors, we validated a truncated CFTR missing a portion of the regulatory domain, CFTR Δ R (1) in an iodide efflux assay, by whole-cell patch-clamp and in a forskolin-induced swelling assay in human intestinal organoids. This demonstrated that CFTR Δ R acts as a functional, cAMP regulated chloride channel and that rAAV-mediated CFTR Δ R gene transfer results in a chloride secretion in human intestinal organoids. Finally, we set out to correct the CF phenotype in Δ F508 mice by nasal instillation of rAAV2/5-CFTR Δ R. rAAV2/5 encoding GFP reporter gene was used as control vector. The therapeutic effect was internally controlled by measuring nasal potential differences (NPD) 1 week before treatment. 2-4 weeks after vector administration, a clear response to low-chloride or forskolin perfusion was observed in 6 out of 8 mice, indicating a restoration of chloride transport across nasal mucosa. Vector-mediated CFTR expression was detected in nose tissue of treated mice, located at the apical membrane of epithelial cells.

CONCLUSIONS: A single administration of rAAV2/5 resulted in sustained reporter gene expression in murine airways, suggesting rAAV2/5 as a promising candidate for a long-term gene therapy for CF. rAAV2/5-CFTR Δ R administration to the airways of CF mice, partially restored chloride conductance across the nasal mucosa as measured by NPD. Our results underscore the therapeutic potential of rAAV2/5-CFTR Δ R for CF gene therapy opening new avenues towards a definitive cure for CF patients irrespective of the underlying mutation.

1. L. S. Ostedgaard *et al.*, *Proc Natl Acad Sci U S A* **102**, 2952 (Feb 22, 2005).

P16

Characterization of the mutations identified in *cis* in F508del complex alleles in the context of corrector therapy and in combination with activators

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Introduction: The identification of small molecules activating the CFTR-G551D channel or correcting the defective routing of CFTR-F508del will enable to propose treatments adapted to the patients genotype. Three different *CFTR* alleles carrying the F508del mutation have been described. The most frequent allele harbours the c.1521_1523delCTT (F508del) mutation alone while two other alleles present an additional mutation in *cis*, either [c.1521_1523delCTT; c.1658G>A] or [c.1521_1523delCTT; c.3080T>C]. These alleles having two distinct mutations are referred to as complex alleles and encode respectively the CFTR-F508del-R553Q and the CFTR-F508del-I1027T channels. They are considered as disease causing as the c.1521_1523delCTT (F508del) mutation is sufficient to be deleterious. We hypothesized that additional defects associated with these mutations could hinder treatment efficiency using correctors alone or in combination with channel potentiators.

The aim of this study is to evaluate the effect of the mutations identified in *cis* of the c.1521_1523delCTT (F508del) mutation in the context of therapies enabling the correction of the trafficking defect of CFTR-F508del and its activation. The identification of defects induced by these mutations in *cis* will enable to predict treatment efficiency and propose an adapted therapeutic.

Methods: In a first step, different *in vitro* assays were combined to evaluate the effect of both c.1658G>A (R553Q) and c.3080T>C (I1027T) on exon skipping, protein maturation and channel function. Splicing defects were evaluated using minigene constructs transfected in Beas-2B cells. CFTR maturation was assessed by Western blot in transiently transfected HEK cells for both single mutants (CFTR-R553Q and -I1027T) and double mutants (CFTR-F508del-R553Q and F508del -I1027T) and function using patch-clamp techniques. Rescue efficiency of the double mutants in response to the VX-809 corrector was compared to CFTR-F508del using both Western blot (WB) and a YFP-based functional assay. Finally, the sensitivity of the mutant channels to the VX-770 potentiators was evaluated.

Results: Minigene results showed that neither c.1658G>A (R553Q) or c.3080T>C (I1027T) altered corresponding exon recognition. Biochemical results showed that R553Q or I1027T mutations did not alter protein maturation; while R553Q reduced channel function (chloride current is halved in R553Q compared to WT CFTR). Double mutants presented a similar profile and function than CFTR-F508del. Rescue efficiency using VX-809 was not modified by the mutation in *cis* as assessed by WB while preliminary results revealed that F508del-R553Q appeared to be less sensitive to combined therapy using VX-809 and VX-770.

Conclusions: This study showed that the I1027T mutation alone or in combination with F508del did not modify CFTR properties. On the other hand, R553Q was shown to reduce channel activity *per se* and channel sensitivity to combinatory therapy using VX-809 and VX-770. These results highlight the importance to fully characterize mutations in *cis* in the context of combinatory CF-therapy.

P18

A LC-MS lipidomics study in lung lavage samples from cf infants reveals differences between severe and mild lung, identifying new therapeutic targets

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Aim: The combined CF clinics Erasmus MC Rotterdam and Utrecht Medical Centers are now engaged in an intensive longitudinal screening protocol, pioneered by the Australian CF community (AREST-CF), in which all CF patients identified by neonatal screening are followed by regular checkups including CT scans, bronchoscopy and collection of lavage fluid (BALF). This provides a unique opportunity to study the early development of CF lung disease and discover novel drug targets. We and others observed abnormalities in the metabolism of bioactive lipids in the lungs of CFTR mutant mice. These molecules are involved in the resolution of inflammation and tissue remodelling. The AREST-CF platform offers a unique opportunity to establish lipid biomarkers in druggable enzymatic pathways, and to monitor intervention studies in infants and pre-school children with CF.

Methods: Using a state of the art LC-MS platform (LACDR Leiden) we have established a method to analyse a panel of oxylipids, polar lipids and fatty acids in 1 ml BALF samples from infants (1-6 years). This technique was applied to 1ml BALF samples obtained from the Australian AREST-CF consortium (represented by Prof Steve Stick et al). The study cohort included samples from CF infants (N=40) with a zero-moderate bronchiectasis score (PRAGMA, Ctvol), and non-CF patients with recurrent inflammation (N=17).

Results: The analysis yielded a matrix of more than ten thousand data points, covering a wide range of bioactive lipids that can be used to monitor inflammation oxidative stress and tissue injury. These data were correlated with lung CT scan scores, and clinical parameters (including neutrophil counts, bacterial load). Cluster analysis shows that we can distinguish CF from non-CF patients particularly in the oxidative stress lipid panel, although neutrophil counts and bacterial load does not differ between the two groups. Importantly, we can distinguish the lipidomics profile of CF patients with severe and mild lung disease (bronchiectasis, bronchial thickening, air trapping, mucus plugging) based on the most advanced CT scan scoring system available (PRAGMA). In particular, receptor activating molecules in metabolic pathways (phospholipase A, sphingosine) previously found to be abnormal in CF mouse lung, were also abnormal in patients with severe lung disease. We are currently investigating the effect of candidate bioactive molecules in a differentiated airway culture system to establish their potential role in the development of CF pathology and options for intervention.

Conclusions: This new approach to monitor the development of CF pathology in individual patients from birth (AREST-CF protocol) offers valuable opportunities for basic and translational research. Correlating biomarkers with progression of pathology allows us to identify new therapeutic targets, monitor the effect of therapeutic interventions and offer treatment tailored to the individual patient.

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P19

The epithelial transcription factor KLF4 affects the traffic of CFTR

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Background: The most frequent mutation F508del, which occurs in ~85% of CF patients worldwide, leads to protein misfolding of the mutant channel which fails to reach the plasma membrane (PM). Due to its misfolded conformation, recognized by the ER quality control, F508del-CFTR is retained in the endoplasmic reticulum and sent for ER-associated degradation. Within an automated microscopy screen aimed at identifying genes affecting the traffic of CFTR in A549 cells, the epithelial differentiation transcription factor Krüppel-like factor 4 (KLF4) was identified as a hit gene. Decreased levels of wt-CFTR traffic to the PM were determined when KLF4 was down-regulated by siRNA

(score = -1.24) suggesting that this epithelial differentiation factor plays a role in CFTR traffic.

Aim: To investigate the expression levels of KLF4 in wt-CFTR and F508del-CFTR stably expressing CFBE cells and to assess the impact of manipulating KLF4 levels on the traffic and function of wt-CFTR and F508del-CFTR.

Methods: The expression levels of KLF4 in stably expressing wt-CFTR and F508del-CFTR CFBE cells were analysed by RT-qPCR, Western blot (WB) and immunostaining. The CRISPR/Cas9 gene editing system was used to knock-out the last two zinc fingers of KLF4 in CFBE cells stably expressing wt-CFTR (CFBE wt-CFTR^{KLF4ΔZf2,3}) and this approach is ongoing for F508del-CFTR expressing CFBE cells. Functional studies of CFTR activity in the above mentioned cells were carried out by patch-clamp.

Results: The KLF4 protein expression levels are significantly lower in cells expressing F508del-CFTR compared to wt-CFTR expressing cells whereas the opposite occurs at the mRNA level. By WB a 3-fold increase of wt-CFTR expression levels are observed in CFBE wt-CFTR^{KLF4ΔZf2,3} cells vs CFBE wt-CFTR cells, indicating that KLF4 affects PM traffic of CFTR. Nevertheless, functional experiments using patch-clamp did not evidence any differences in CFTR activity between the two cell lines. On the other hand, and consistently with the WB data in CFBE wt-CFTR^{KLF4ΔZf2,3} cells vs CFBE wt-CFTR cells, overexpression of KLF4 in CFBE wt-CFTR expressing cells results in a reduced activation of CFTR by IBMX/FSK. Further studies of KLF4 knock-down (siRNA) and KLF4 overexpression (cDNA) on wt- and F508del-CFTR as well as studies on polarized wt- and F508del-CFTR expressing CFBE cells are currently ongoing.

Conclusion: In summary, our results suggest that KLF4 plays an important role in CF since basal expression levels of KLF4 protein are significantly reduced in CFBE F508del-CFTR cells. The data further indicates that KLF4 acts on the traffic of wt-CFTR to the PM suggesting it to be a target to help rescue F508del-CFTR to the cell surface.

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P20

Defective PPARg signaling and increased mucin/bicarbonate expression in the regulation of human airway epithelial cell repair

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Cystic fibrosis (CF) is caused by mutations in the CFTR apical chloride channel which supports bicarbonate transport. Recurrent infection and inflammation of the CF airway epithelium causes continuous repair which may lead to remodeling of the normal pseudostratified mucociliated tissue. Direct cell-to-cell interactions are critical in coordinating repair and in the transition of repairing cells from a self-renewal, multipotent stage to an early differentiated stage. Although there is evidence that wound repair is defective in CF, the underlying mechanisms regulating this process are poorly known. Here, we monitored the gene expression changes of Notch and peroxisome proliferator-activated receptor g (PPARg) in a 3D human airway epithelium wound repair model. These pathways are involved in determining cell fate and specific lineages of undifferentiated progenitor epithelial cells. We have previously shown that the gap junction protein connexin26 (Cx26) is expressed in a cytokeratin14 (KRT14)-subpopulation of progenitor cells capable to re-differentiate into all three main cell types of the airway epithelium. Human airway epithelial cells (HAECs) from non-CF (NCF) and from CF patients were differentiated at the air/liquid interface. The cells were mechanically wounded and the expression and/or localization of transcription factors and Cxs were monitored by qPCR and immunofluorescence (IF) in repairing HAECs, 24hrs after wounding, when the ablated area was covered (wound closure; WC) and at 10, 20 and 30 days after WC. Hes1 gene expression was used to monitor the activation of Notch signaling pathway during repair. Hydroxyprostaglandin dehydrogenase 15-(NAD) (HPGD)- the enzyme providing PPARg's endogenous ligand 15-ketoprostaglandin E2 (15kPGE2)- expression was measured to follow PPARg pathway. Moreover, we measured the expression of MUC5AC, MUC5B and carbonic anhydrase 2 and 12 (CA2 and CA12). Ciliated cell markers (FOXJ1 and DNAI1) transiently decreased during wound repair while markers of progenitor cells (KRT5 and KRT14) increased, validating our model. We found that Hes1 expression was unaltered during repair in CF cells as compared to NCF cells. In contrast, HPGD expression was lower in CF cells as compared to NCF cells ($p < 0.05$, $N=9/11$) in non-wounded (NW) cultures and after 30 days of repair ($p < 0.05$, $N=4/6$). Similarly, the expression of PPARg was also reduced 30 days after repair in CF cells as compared to NCF cells ($p < 0.05$, $N=4/6$). Interestingly, MUC5AC expression was reduced in CF NW cultures ($p < 0.05$, $N=6/4$). Moreover, MUC5B and CA2 expression, but not CA12, was significantly increased in repairing CF cells at 24hrs after wounding and at WC, as compared to NCF cells ($p < 0.05$, $N=4/6$). In both NCF and CF cells, wounding triggered progenitor cell proliferation and Cx26 expression increase, which returned to basal levels after WC. Cx26 plaque counts revealed by IF was markedly decreased upon PPARg activation with 15-kPGE2 while PPARg inhibition with its pharmacological inhibitor GW9662 increased Cx26 plaque counts ($p < 0.05$, $N=4$). Our data show a deregulation of PPARg pathway in CF cells that may affect the repair process by changing Cx26-mediated cell-cell interactions and mucus cell differentiation. Experiments are underway to determine the causal relationships between CFTR, PPARg signaling and mucin/bicarbonate production.

P21

Orphan G-protein coupled receptor CELSR3 is an epithelial marker downregulated in cystic fibrosis

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Airway epithelial barrier function is impaired in cystic fibrosis and this phenomenon has been linked to defective trafficking of F508del-CFTR. In addition, abnormal expression and localization of CFTR, even wild-type protein, may also be observed during epithelial remodeling and dedifferentiation. In both situations, the role of apico-basal polarity is crucial. Epithelial cells also exhibit Planar Cell Polarity (PCP) which has been described in mammal lung epithelial cells as a crucial mechanism controlling coordination, development and function of epithelial specialized structure in the plane of the epithelium. However, little is known about PCP in bronchial epithelial cells.

We demonstrated that human bronchial epithelial cells expressed several PCP core and effector proteins. In particular, we demonstrated that atypical cadherin CELSR3 (Cadherin EGF LAG seven-pass G-type receptor 3) was expressed in primary HBEs and in HBE cell lines, and its expression was downregulated in CF (F508del/F508del)-HBEs as compared to non-CF cells. CELSR3 belongs to the G-Protein Coupled Receptor (GPCR) protein family and to the adhesion GPCR subfamily. These particular GPCRs are characterized by a very long extracellular N-terminus containing among others several cadherin domains suspected to establish homophilic interactions between neighboring cells to mediate cell-cell interactions. To confirm the role of CELSR3 in epithelial polarity, we induced epithelial mesenchymal transition (EMT) in 16HBE monolayers with Transforming Growth Factor beta (TGF β) or cisplatin (CIS). Both molecules induced concomitant loss of CELSR3 and epithelial marker ZO-1 and gain of fibrotic markers vimentin and fibronectin. We also demonstrated that knocking down expression of CELSR3 in 16HBE cells enhanced TGF β -induced EMT. However, knocking-down CELSR3 did not modulate transepithelial electric resistance (TEER) i.e. epithelial barrier function, showing that CELSR3 is an epithelial marker but not a direct determinant of intercellular junction tightness.

Taken together, our results demonstrated that CELSR3 is an epithelial marker down-regulated in cystic fibrosis bronchial epithelial cells. Moreover, loss of CELSR3 function may confer profibrotic phenotype to CF cells.

P23
Role of miRNAs in the CF pathology

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Our group is interested in the identification of regulatory elements, including transcription factors and miRNAs, that control the *CFTR* mRNA level.

We recently characterized miRNAs acting only in lung adult cells compared to data from primary fetal cells. Our finding allowed us to envision new therapeutic tools focused on miRNAs. We used an experimental model based on scratching of nasal epithelium from healthy individuals or patients with cystic fibrosis and homozygous for the p.Phe508del *CFTR* mutation, cultured in an air-liquid interface (ALI). This model allows testing molecules for the correction of *CFTR*-mutation consequences. We demonstrated that introduction of oligonucleotides (which specifically block the binding of miRNAs) increased the *CFTR* mRNA and *CFTR* protein levels on CF nasal epithelium and enhanced *CFTR* function in CFBE14o- cells*.

It is becoming increasingly clear that in addition to their role in the *CFTR* mRNA expression, miRNAs play a role in manifestations of CF lung disease. For instance, miR-101 and miR-145 are deregulated in vivo in CF bronchial epithelium.

Material and methods: To identify other putative targets, we assessed the gene expression pattern of miRNAs in ALI epithelium cultured from CF patients (p.Phe508del homozygous) or healthy. We compared two approaches i.e. miRNA sequencing (miRseq by using MiSeq, Illumina) and TLDA system (TaqMan Low Density Array) and three models i.e. ALI epithelium from nasal, polyps or bronchial biopsies (n=4 per condition). For miRseq, three different programs were applied including MiSeqReporter, MiRanalyzer and sRNAbench.

Results: First results confirmed the high expression of the miR-449 family in ALI models previously described as key regulators ciliogenesis. In addition, these miRNAs are over-expressed in CF versus non-CF in two models. We also observed the deregulation of miR-101 and miR-27b in CF polyps and CF bronchial ALI. Treatment of ALI epithelium with inhibitors for some miRNAs is being tested. Role and targets of some deregulated miRNAs are also in progress.

Conclusion: Finding new regulatory players involved in CF physiopathology and/or controlling the *CFTR* mRNA level help us to envision new tools for Cystic Fibrosis therapy.

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- Transcription factors and miRNAs that regulate fetal to adult *CFTR* expression change are new targets for cystic fibrosis, Eur Respir J.2015 Jan; 45(1):116-28

P24

MiR-210 and miR-155 are upregulated in cystic fibrosis cells and involved in Fe-S protein assembly via ISCU downregulation as well as hemoxygenase-1 expression via BACH1

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Cystic fibrosis (CF) is an inherited lung disorder associated with chronic endobronchial inflammation. Hemoxygenase-1 (HMOX1), an enzyme involved in heme degradation, is upregulated in inflammation and negatively regulated via the transcription factor BACH1. Here we studied the role of microRNAs (miRs) in the heme metabolism of cystic fibrosis bronchial epithelial cells (CFBE41o-), applying qRT-PCR, luciferase promoter assays, immunoblotting, and enzyme activity assays. In comparison to normal human bronchial epithelial cells (16HBE14o-) miR-210 and miR-155 were 4- to 5-fold upregulated in CFBE41o- cells. MiR-210 has been implicated in the negative regulation of the mitochondrial iron-sulfur (Fe-S) cluster assembly scaffold ISCU, and consistently we found ISCU expression being decreased in CF cells. Accordingly, activities of the Fe-S enzymes aconitase, succinate dehydrogenase, and ferrochelatase (FECH) were also diminished. Despite this latter constraint total heme content was higher in CF than in control cells. Intriguingly, HMOX1 expression in CF cells was severely curtailed, implying that despite the low FECH activity heme formation was in favour of heme degradation. Moreover, we could link HMOX1 expression to TLR4 surface expression via miR-155 which in turn negatively regulates the expression of BACH1. Since TLR4 expression is impaired in CF we applied an TLR4 inhibitor in normal HBE cells and found levels of both miR-155 and BACH1 increased in a dose-dependent manner. Hence our results suggest that in CF miR-155 is ineffective in repressing BACH1, leading to low HMOX1 levels and altered heme metabolism, while miR-210 affects cellular Fe-S assembly with potential general impact on cellular iron homeostasis.

P25

Differential gene activation in normal and cystic fibrosis human bronchial epithelial cells upon *Pseudomonas aeruginosa* infection

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Rationale: Cystic fibrosis (CF), a genetic disease caused by mutations in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene, is characterized by airway colonization by *Pseudomonas aeruginosa* (*P.a*) resulting in a prolonged inflammatory response. Airway epithelium plays an important role in the innate defense by sensing microorganisms that leads to the synthesis of chemokines and cytokines that orchestrate leucocytes recruitment and activation and secretion of antimicrobial peptides. In the present study, we performed transcriptomic analysis of *P.a*-infected primary bronchial epithelial cells from healthy donors and CF patients. The comparative analysis of mRNA expression profiles between CF and non-CF cells may help to understand how *P.a* differentially initiates infection and to explain its persistence in lungs in spite of a strong inflammatory response.

Methods: Human primary bronchial epithelial cells expressing a functional (non-CF) or mutated (CF) CFTR (F508Del homozygous) were infected by *P.a* (PAK strain) at 0.25 MOI for 0, 2, 4 and 6 hours. For each time point, biological quadruplicates (4 donors per group, non-CF and CF) were processed for mRNA profiling (a total of 32 samples) using Illumina mRNA sequencing. Statistical analysis provided lists of genes having a significant differential expression ($p < 0.001$) between the CF and non-CF at 0, 2, 4 and 6 hours of infection.

Results: We found 310, 295, 252 and 448 genes differentially down-regulated and 541, 343, 415, 532 differentially up-regulated in CF compared with non-CF cells at 0, 2, 4 and 6 hours of infection, respectively.

We identified differentially up-regulated genes with biological plausibility that could explain the intense inflammatory response and differentially down-regulated genes whose reduced expression of products could explain the development of chronic infection and the absence of anti-inflammatory response of infected CF patients. Several genes such as IL-6, IL-17C, KLF2, TLR4, SLPI or GSTT1 were already known to play a role in the epithelium-*P.a* interaction or in CF but interestingly some of the dysregulated genes were never or sparsely described in the lung CF disease.

Conclusions: The link between the worsening in CF and infection with *P.a* is well established. Different clinical approaches are used to eliminate *P.a* especially antibiotic prescriptions, but none of them leading to complete eradication. Here, we identified mRNA pattern associated with *P.a* infection that could explain the lack of *P.a* eradication in CF. This transcriptomic analysis may be the first step to study the development of new diagnostic tools and therapeutic strategies.

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P26

***Pseudomonas aeruginosa* adaptation to cystic fibrosis airways shapes the host response in mice during the progression of airway disease**

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Background: Repeated cycles of infections, caused principally by *Pseudomonas aeruginosa*, combined with robust host immune response determine the course and outcome of respiratory disease in cystic fibrosis (CF). During the course of chronic colonization *P. aeruginosa* adapts to the airways and dramatically modifies its phenotype promoting evasion of the host immune recognition, as described previously. However, structural changes including degradation of elastin, collagen deposition and high level of glycosaminoglycans (GAG) and matrix metalloproteinases (MMP) are associated to the progression of lung disease in CF patients.

Objectives: It is still not clear whether and how the *P. aeruginosa* adaptation to CF airways affects the host immune response and favors airway damage and the remodeling processes leading to lung decline.

Method: For this study, we used a clonal lineage of *P. aeruginosa* strains sampled at the onset of infection and after 7.5 years before the patient died; they were selected for their diversity determined in previous characterization. These bacterial strains were challenged in C57Bl/6 and CF ko mice after their inclusion in agar beads to establish long-term chronic lung infection. Mice were evaluated for bacterial count, lung histopathology and markers of inflammation and tissue damage.

Results: Following the peak of infection, early *P. aeruginosa* strain embedded in agar-beads either disseminated systemically and induced death, or was cleared by the host, while CF-adapted variants persisted for up to three months in a set of mice (~30%) with a bacterial load stabilized at $\approx 10^4$ CFU/lung. Observing the murine host response over the course of the airways infection, CF-adapted variants resulted in lower recruitment of innate immune cells and production of cytokines/chemokines involved in leukocytes recruitments (day 28) compared to the early phase (day 2), while levels of IL-17 and IFN- γ , associated to lymphocyte polarization, remained high. The long-term consequence of CF-adapted variants is the implication of an adaptive immune response and the formation of bronchus-associated lymphoid tissue (BALT)-like structures, as indicated by histological scores. Immunohistochemistry with anti-CD3 and B220 antibodies showed that BALT structures were consisted mainly of T and B cells, confirming a shift in the subtypes of immune cells recruited during the course of chronic infection. Hallmarks of airway remodelling and fibrosis, including epithelial hyperplasia and structure degeneration, goblet cell metaplasia, collagen deposition and elastin degradation were also observed by the pathological scores. MMP-9 activity and protein, TGF- β and sGAG were detected and increased over time during the long-term infection in murine lungs infected with the CF-adapted variants. When long-term chronic infection was established in CF mice, we found higher goblet cell metaplasia in comparison to wt mice, but no differences for other markers.

Conclusions: We conclude that *P. aeruginosa* adaptation plays a significant role in the progression of airway disease shifting the host response toward airway remodeling and tissue damage. In addition, CF-adapted variants are critical success determinant for reproducing the human disease in mice.

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P27

Dissection of host susceptibility to *Pseudomonas aeruginosa* lung infection in mice

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The progression and severity of *P. aeruginosa* infection do not appear to correlate with the type of *CFTR* variant and rather seems to be largely dependent on other factors, which remain to be established. Generally, host susceptibility to infectious diseases including to *P. aeruginosa* are complex traits that need to be analyzed to understand the full repertoire of host response to this pathogen. To map host genes underlying such complex diseases, well-defined mouse genetic reference populations of inbred mice have been a powerful tool. As a first step towards establishing mouse mapping resource population, we assessed nine classical inbred strains of mice (A/J, BALB/cJ, BALB/cAnNCrI, BALB/cByJ, C3H/HeOJ, C57BL/6J, C57BL/6NCrI, DBA/2J, and 129S2/SvPasCRL) for the susceptibility to *P. aeruginosa* clinical strains infection. Recently, we demonstrated that the genetic makeup of the host influences the response to infection (De Simone *et al*, PlosOne 2014). In particular, A/J and C3H/HeOJ showed the most deviant clinical (e.g. body weight, mortality, chronic infection) and immunological phenotypes (e.g. cytokines response, innate and adaptive cell response). Based on these results, susceptible A/J (mean survival time of 0.5 days) and resistant C3H/HeOJ (mean survival time of 3.8 days) mice were crossed to generate 400 F2 population, and subsequently used to map host genetic determinants of susceptibility to *P. aeruginosa*. All F2 mice were challenged with *P. aeruginosa* clinical strain, and monitored for mean survival time up to seven days post infection, as disease phenotype associated trait. In particular, a significant different mean survival time ranging from 0.5 to 7.0 days was observed in F2 mice population compared to the parental lines. Thus, the phenotypic extremes (20% high susceptible and 20% most resistant) of F2 population were genotyped with about 10K informative single nucleotide polymorphic (SNP) markers, and subsequently performed quantitative trait loci (QTL) mapping. We identified a significant QTL on chromosome 6 at 90.8 Mbp with 4.3 LOD Score, with a 1.5-LOD drop support interval of 81.5-102.2 Mbp. A total of 327 genes within the mapped locus were identified from the public database ([http:// www.informatic.jax.org/](http://www.informatic.jax.org/)), and classified in predicted proteins, RNA genes, heritable phenotypic markers and non-coding RNA genes. Gene prioritization approach by specific computational tools (Endeavour program) and score analysis revealed few genes possibly involved in susceptibility to *P. aeruginosa* infection : *Dok1*, *Tacr1*, *Cd207*, *Clec4f4*, *Gp9*, *Foxp1*, *Gata2* and *Wnt7a*, principally involved in pathogen sensing, neutrophils and macrophages recruitment, inflammatory cytokines expression and epithelial alveolar turnover process. Further characterization of these genes may lead to the identification of novel modifiers involved in the pathogenesis of CF and may complement human studies.

De Simone, M., L. Spagnuolo, et al. (2014). "Host genetic background influences the response to the opportunistic *Pseudomonas aeruginosa* infection altering cell-mediated immunity and bacterial replication." PloS one 9(9): e106873.

P28

Identification of metalloprotease activity in *P. aeruginosa* clinical strains and its sensitivity to azithromycin treatment through a metalloprotease activity assay based on flow cytometry

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Background and aims: Modulation of *Pseudomonas aeruginosa* virulence factors was suggested as a mechanism for azithromycin (AZM) beneficial effects in CF patients. Members of the metalloprotease family of enzymes (MPs) are released by *P. aeruginosa* chronic and sporadic clinical strains and regulated by AZM. We analyzed MPs activity on culture supernatants from *P. aeruginosa* clinical strains by gelatin-zymography, an assay for detection and quantification of protease activity. Nonetheless it has a low dynamic range, it is time consuming and hardly suitable for routine analysis. Our work aimed to develop a highly reproducible and easy method to detect MP activity in *P. aeruginosa* clinical strains and to evaluate possible effects of AZM.

Methods: This method based on the flow cytometric assay with fluorochrome-labeled substrate

(FITC-gelatin) coated microspheres allowed to evaluate MP activity in supernatants (SNs) from sporadic or chronic strains isolated from the airways of CF patients. We have investigated MP activity in SNs derived from 136 bacterial isolates classified as sporadic and 134 defined as chronic.

Results: MP activity was detected in 100 of 136 of the sporadic (73%) but only in 70 of 134 (52%) of the chronic strains ($p < 0.0001$, Fisher's exact test). AZM treatment induced a decrease of MP activity in 76 of 91 (83%) of the sporadic strains but only in 28 of 56 of the chronic strains releasing MPs (50%)

($p < 0.0001$, Fisher's exact test). We detected *Pseudomonas*-derived AprA MP in the sputa of CF patients. When we tested the assay in the cell-free sputum from chronically colonized CF patients untreated or treated with AZM ($n=37$) a similar decrease of the fluorescent signal on gelatin-coated beads was detected in both conditions (76 % reduction average based on MFI values, for $n=14$ untreated patients, 75% reduction for $n=23$ AZM- treated patients) suggesting that the presence of leukocyte-derived proteases mask the contribution of the bacterial-derived ones.

Conclusions: The presence of metalloprotease activity in the cell-free sputa could suggest that bacterial MPs are indeed released by *P. aeruginosa* in the lung microenvironment and might contribute, along with leukocyte-derived enzymes, to lung damage in CF patients. The method described is sensitive and reproducible and has a high dynamic range. In the absence of more specific substrates capable to differentiate specific MPs this assay appears suitable to measure total MP activity nevertheless, using isolated microorganisms, it is possible to document variation in MP release and regulation.

P29

The role of mucus and mucin in mediating *Pseudomonas aeruginosa* infection and virulence in cystic fibrosis

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Pseudomonas aeruginosa colonises the mucus in the lungs of individuals with cystic fibrosis (CF). The prevention of chronic *P. aeruginosa* infection is a cornerstone of clinical care in CF. In this study we hypothesise that the environment of the CF lung, which contains thick stagnant mucus and mucins with altered glycans compared to non diseased individuals plays an important role in initiation of colonisation and maintenance of chronic bacterial infection. To test this hypothesis we have characterised the interaction of *P. aeruginosa* isolates with a variety of respiratory mucus secreting and non mucus secreting cell lines. Binding of *P. aeruginosa* strain PAO1 and strains from CF and non CF patients to mucins purified from cell lines and from different human and animal anatomical sites was assessed using a novel mucin microarray platform. Neo-glycoconjugates printed on a microarray were also probed with *P. aeruginosa* strains in order to identify potential inhibitors of initial colonisation. The effect of mucus on biofilm formation and antibiotic sensitivity was also assessed.

Infection assays showed that while *P. aeruginosa* strains colonised mucus secreting and non-mucus secreting respiratory cells, it displayed a predilection for mucus on the surface of cells. In addition bacterial aggregates were observed in cells of CF and non CF origin that formed adherent mucus layers. Higher numbers of bacteria were found to colonise a CF mucus secreting cell line than colonised non CF mucus secreting cells.

Strong binding of CF *P. aeruginosa* isolates and strain PAO1 to mannose, glucose, galactose, fucose and to the blood group antigens Lewis^x, Lewis^b, and H2 was detected. Differences in binding to sialyl Lewis^x, Lewis^y and 3' and 6' sialyl lactose was observed between strains. Similar binding profiles were seen for strains grown to log and stationary phase. The strength of the interaction of *P. aeruginosa* with mucins was not as strong as that seen with individual glycans. Binding of *P. aeruginosa* to native mucins from human and animal sources depended on the bacterial strains and the source of mucin suggesting that strains express different mucin binding adhesins. In contrast strains exhibited a very similar binding profile to mucins from cell lines with less complex truncated mucins compared to native mucins.

P. aeruginosa exhibited strong binding to the human blood group antigen, Lacto-N-fucopentose I, and incubation of *P. aeruginosa* with Lacto-N fucopentose I inhibited bacterial binding to both mucus secreting and non-mucus secreting cells. Growth of *P. aeruginosa* in the presence of mucin promoted both biofilm formation and antibiotic resistance.

The reasons for the particular predilection of *P. aeruginosa* for the CF airway, and the very significant negative clinical implications of colonisation are incompletely understood. Together these results suggest that mucus promotes both infection and virulence of *P. aeruginosa*. The identification of both bacterial and host factors that mediate infection with *P. aeruginosa* in CF patients should lead to the development of new therapeutic strategies to prevent disease.

P30

Azithromycin effect on lung inflammation induced by *Pseudomonas aeruginosa* released proteases as shown by in vivo imaging in IL-8 transiently transgenized mice

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Azithromycin (AZM) is utilized in the treatment of chronic inflammatory diseases like diffuse panbronchiolitis, cystic fibrosis (CF) and COPD also for evidences of effects in contrasting lung inflammation, although the mechanisms are still under investigation. Recently, a mouse model has been developed transiently expressing the luciferase reporter gene under the control of an interleukin 8 (IL-8) bovine promoter (bIL-8 luc).

In the present study, the transgenic mouse has been used to in vivo observe IL-8 mediated lung inflammation induced by *Pseudomonas aeruginosa* secreted virulence factors, specifically metalloproteases (MP) and to test the possible anti-inflammatory effect of AZM. Culture supernatants (SN) from two *P. aeruginosa* strains (VR1; VR2), isolated from the sputum of sporadically infected CF patients, were obtained after growth in the absence or presence of a sub-MIC dose of AZM (\pm AZM). SNs were analyzed for the presence of MPs by using smart fluorescent probes that can be activated by proteases. VR1-AZM showed metalloprotease, pan-cathepsin and elastase activities while VR1+AZM lost them. VR2 \pm AZM SNs showed no protease activity. In vivo monitoring of lung inflammation after stimulation with *P. aeruginosa*

SNs has been conducted by imaging of bIL-8 luc transgenic mice. The pro-inflammatory activity of VR1-AZM was clearly visible 24 hours after the instillation while a significant decrease of this response was observed in presence of VR1+AZM supernatant. On the contrary, VR2-AZM did not induce any measurable inflammation in the mouse lung. BALF was recovered from IL-8 transgenic mice in order to evaluate the effect of VR1 and VR2 \pm AZM SN on inflammatory cell recruitment. VR1-AZM SN, containing MPs, stimulated recruitment of total cells and neutrophils while it was reduced after VR1+AZM challenge. Moreover, a selected panel cytokines resulted up-regulated after VR1-AZM instillation while their expression was lower in presence of VR1+AZM: IL-1 beta, IL-17, IL-12(p70), RANTES, KC. The VR2-AZM SN had an effect 4-fold lower as concern inflammatory cells recruitment.

These data suggest that bacterial proteases might be a target of AZM. The in vivo model here used for monitoring bacteria-induced inflammation might be applied to study pathogenesis of diseases such as cystic fibrosis and to evaluate the possible therapeutic effect of known and new molecules with a presumptive anti-inflammatory action.

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***Pseudomonas aeruginosa* elastase LasB modulates CFTR expression, innate immune responses and epithelial repair in airway epithelial cells**

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Introduction: *Pseudomonas aeruginosa* (*P.a.*) is an opportunistic pathogen that is responsible for nosocomial infections and is the major *bacterium* found in the Cystic Fibrosis (CF) lungs. Our group has shown that host endogenous proteases such as neutrophil elastase are able to cleave and inactivate CFTR in the airways¹. Here we hypothesize that the *P.a.* virulence factor elastase LasB could modulate CFTR expression and innate immune responses in airway epithelial cells.

Methods: The effect of recombinant LasB and secretomes from *P.a.* strains (WT and Δ LasB PAO1, referred as WT-SEC and Δ LasB-SEC) on CFTR integrity was evaluated in the epithelial cell line NCI-H292 infected with the adenovirus Ad GFP-WT-CFTR and in the Calu-3 cell line grown on semi-permeable supports. The effect of LasB on ion transport was measured using the corrected CF cell line CFBE41o-WT-CFTR in Ussing chambers experiments. Epithelial repair and cytokinic and anti-microbial peptides (eg secretion of elafin) profiles were evaluated in the NCI-H292 cell line after treatment with LasB or the secretomes.

Results: SDS-PAGE analysis of the secretomes showed that LasB is the main secreted protein by WT PAO1. Recombinant LasB (10-25 nM) and the WT-SEC (1-10%) but not the Δ LasB-SEC, decreased CFTR expression. WT-SEC (10%) significantly decreased CFTR activity as reported by the measurement of forskolin/ibmx induced short-circuit current (Δ Isc, $20.7 \pm 10.3 \mu\text{Amp.cm}^{-2}$) and CFTR_(Inh-172)-sensitive Δ Isc, when compared to the effect of the Δ LasB-SEC ($30.0 \pm 9.7 \mu\text{Amp.cm}^{-2}$) or control condition ($28.1 \pm 10.4 \mu\text{Amp.cm}^{-2}$) ($p < 0.01$, ANOVA, $n=3$). Finally recombinant LasB, as well as the WT-SEC but not Δ LasB-SEC abolished IL-1 β -induced IL-6 increase (rec. lasB: $96.9\% \pm 0.6$ decrease, $p < 0.01$, $n=4$, WT-SEC: $92.8\% \pm 0.8$ decrease, $p < 0.001$, $n=4$ and Δ LasB-SEC: $8.2\% \pm 6.9$ decrease, $p > 0.05$) as well as IL-1 β -induced elafin secretion (an important epithelial-derived antimicrobial molecule, rec. lasB: $93.4\% \pm 3.0$ decrease, WT-SEC: $96.2\% \pm 1.3$ decrease, $p < 0.001$, $n=4$ and Δ LasB-SEC: $-1.9\% \pm 7.9$ decrease, $p > 0.05$). Interestingly, LasB did not have any effect on IL-8 secretion. WT-SEC also inhibited basal and IL-6-induced epithelial repair in scratch assay experiments (basal conditions: WT-SEC: $59.8\% \pm 16.2$ decrease, $p < 0.01$, $n=5$, 2-way ANOVA; IL-6: WT-SEC: 74.7 ± 5.5 decrease, $p < 0.001$, $n=5$, 2-way ANOVA).

Conclusion: Taken together, our results show that LasB, the major Type 2 Secretion System virulence factor present in *P.a* secretome, is able to modulate the expression of CFTR and its activity. Moreover, we show that this factor targets the immune response by regulating the expression of cytokines and

anti-microbial peptides. We are currently investigating the molecular mechanisms and signalling pathways underlying these processes.

1. Le Gars et al. Am. J. Respir. Crit. Care Med. 2013 187(2) :170-9

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P32

The role of lipoxin A₄ on tight junctions and antimicrobial peptide production by CF airway epithelial cells

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In Cystic Fibrosis (CF), mutations of the CF gene (CFTR), which encodes for a chloride channel, results in defective Cl⁻ secretion and Na⁺ hyperabsorption in the airway. This ion transport dysfunction contributes to a reduction of the airway surface liquid (ASL) height, impairs mucociliary clearance, thus favoring bacterial colonization and inflammation. We have recently shown that lipoxin A₄ (LXA₄) a mediator of the resolution of inflammation, which is abnormally produced in CF, restored the ASL height. Here, we examined the effect of LXA₄ on bacterial invasion and started to identify the cellular mechanism involved. Exposure of *P. aeruginosa* to bronchial epithelial cells decreased the amount of the tight junction protein ZO-1 and disrupted tight junctions formation. In contrast, LXA₄ increased ZO-1 mRNA and protein synthesis and prevented the decrease of ZO-1 protein and tight junction disruption induced by *P. aeruginosa*. Furthermore, LXA₄ stimulated the production of the antimicrobial peptide Human beta-defensin-2 (hBD-2) by the airway epithelial cells. Finally, using an adhesion assay and confocal microscopy techniques, we found that LXA₄ delays invasion of CF bronchial epithelia by a clinical strain of a CF *P. aeruginosa* and by the laboratory strain PAO1. Taken together, these novel effects of LXA₄ on airway epithelium may open new therapeutic avenue for CF lung disease.

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Lipoxin A₄ regulates cAMP dependent ion transport and cell migration in the colonic cell line T84

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Lipoxin A₄ (LXA₄) is an endogenous lipid mediator described for its beneficial function during the resolution of inflammation. Mostly LXA₄ displays its actions by binding to the G-protein coupled formyl peptide receptor 2 (ALX/FPR2). Due to its role as an inflammation resolving molecule, LXA₄ is associated with beneficial effects in numerous diseases in which inflammation may occur, such as inflammatory bowel diseases, colitis, inflammation associated colon cancer and also cystic fibrosis. These diseases are characterised by epithelial ion transport abnormalities. However, the impact of LXA₄ on ion transport, cell migration and proliferation in the colonic epithelium is not known.

In order to examine the impact of LXA₄ on transepithelial ion transport, colonic T84 cells were grown in monolayers on filters and transepithelial ion current was recorded in Ussing chambers. Additionally, the expression and localisation of the ALX/FPR2 receptor in the human colonic cell lines HT29Cl.19A and T84 was investigated by western blot, FACS (fluorescence activated cell sorting) and immunofluorescence analysis. For the investigation of colonic cell migration a Boyden chamber assay was used and cell proliferation was assessed with an MTT assay.

The LXA₄ receptor, ALX/FPR2, was expressed in T84 and HT29Cl.19A colonic cells and LXA₄ stimulated ALX/FPR2 receptor expression in these cells. Apical LXA₄ (1 nM, 10 nM or 100 nM) did not influence either the baseline transepithelial ion transport or the ion transport increase induced by the calcium ionophore A23187 (2 µM, apical). However, apical LXA₄ (100 nM) significantly reduced the current increase evoked by forskolin (10 µM, apical), suggesting that LXA₄ inhibits cAMP-dependent ion currents in colonic cells. Furthermore, LXA₄ (1 nM, 10 nM or 100 nM) reduced basal and forskolin-induced cell migration as well as basal proliferation.

In conclusion, we have shown that LXA₄ decreases the cAMP-dependent ion transport as well as cell migration and proliferation of colonic cells. Identification of the ion channels involved in this response and the effect of LXA₄ in CF intestinal epithelium is currently under investigation.

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Airway epithelial cell integrity protects from cytotoxicity of *P. aeruginosa* quorum-sensing signals

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Rationale: Cell-to-cell communication via gap junctions regulates airway epithelial cell homeostasis and maintains the epithelium host defense. Quorum-sensing molecules produced by *Pseudomonas aeruginosa* coordinate the expression of virulence factors by this respiratory pathogen affecting cystic fibrosis patient. These bacterial signals may also incidentally modulate mammalian airway epithelial cell responses to the pathogen, a process called inter-kingdom signaling.

Objectives: To investigate the interactions between the *P. aeruginosa* N-3-oxo-dodecanoyl-L-homoserine lactone (C12) quorum-sensing molecule and human airway epithelial cell gap junctional intercellular communication (GJIC).

Methods: C12 degradation and its effects on cells were monitored in various human airway epithelial cell models grown under non-polarized and polarized conditions. Its concentration was further monitored in daily tracheal aspirates of colonized intubated patients.

Measurements and main results: C12 rapidly altered epithelial integrity (inducing shrinkage and blebbing) and decreased GJIC in non-polarized airway epithelial cells while other quorum-sensing molecules had no effect. C12 also increased $[Ca^{2+}]_i$ by promoting calcium release from the ER and calcium influx. The cytotoxic effects of C12 were dependent on $[Ca^{2+}]_i$ and could be prevented by inhibitors of Src tyrosine family and Rho-associated protein kinases. In contrast, polarized airway cells grown on Transwell filters were protected from C12, except when undergoing repair after wounding. *In vivo* during colonization of intubated patients, C12 did not accumulate, but paralleled bacterial densities. Interestingly, *in vitro* C12 degradation, a reaction catalyzed by intracellular paraoxonase 2 (PON2), was impaired in non-polarized cells whereas PON2 expression was increased during epithelial polarization. Finally, in a wound injury model of primary airway epithelial cells grown at the air-liquid interface, repeated exposure of C12 impairs airway epithelial cell repair.

Conclusions: The cytotoxicity of C12 on non-polarized epithelial cells, combined with its impaired degradation allowing its accumulation, provide an additional pathogenic mechanism for *P. aeruginosa* infections. The lung of cystic fibrosis patients chronically infected with *P. aeruginosa* is characterized by extensive tissue injury and high concentration of C12. Therefore, the development of drugs or therapies targeting C12 or enhancing PON2 activity might be helpful for fighting *P. aeruginosa* infections in cystic fibrosis.

P35

A new synthetic compound inhibits the expression of Quorum-Sensing regulated genes and biofilm formation in *Pseudomonas aeruginosa*

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Objectives: *Pseudomonas aeruginosa* is a ubiquitous bacterium that can cause severe and chronic lung infections in patients with cystic fibrosis, particularly in establishing a structured form called biofilm, which is very difficult to eliminate by antibiotics. It has been shown that the bacterial Quorum-Sensing (QS) communication system play a key role in the development of biofilms and in orchestrating the expression of many *P. aeruginosa* virulence factors. *P. aeruginosa* possesses two complete QS systems, *rhl* and *las*, which are mainly controlled by two natural *N*-acyl homoserine lactones (HSL) signal molecules, C4-HSL and 3-oxo-C12-HSL respectively. Thus, the aim of our study is to design potent HSL analogs and to screen them for their antibiofilm activity and their effect on QS-regulated genes expression.

Methods: Various analogs were designed on the basis of the C4-HSL structure, by replacing the lactone ring, or the aliphatic chain, by groups modulating the electronic effects or the steric hindrance of the molecules. These compounds were synthesized and screened for their ability to impair biofilm formation in an innovative *in vitro* model developed in the laboratory. In this model, analogs effectiveness was evaluated by CFU enumerations of adherent cells and confocal microscopy observations. In parallel, the quantitative real-time RT-PCR was developed to analyze the effects of analogs on the expression of some QS-regulated genes.

Results: Among the analogs, a compound called C11 (N-pyrimidyl butanamide) showed a significant inhibition of biofilm formation in a dose-dependent manner, coupled with an absence of cytotoxicity on lung cells. C11 was then tested in combination with antibiotics and a significant synergistic antibiofilm effect was obtained with ciprofloxacin, tobramycin and colistin. Moreover, this inhibitory activity of C11/antibiotics combination was preserved on a biofilm developed under anaerobic conditions, set up to approximate the *in vivo* colonization conditions by the bacterium. Moreover, a significant down-regulation of QS-regulated genes by C11 was observed, both for genes regulating the *rhl* system (*rhlR* and *rhlI*) as the *las* system (*lasR* and *lasI*). After a structure-activity study performed on C11, new analogs were synthesized and three showed a significant antibiofilm activity.

Conclusion: The combination of C11 with antibiotics showed significant inhibitory effects on the biofilm development and the QS-regulated gene expression in *P. aeruginosa*. Different combination of analogs with antibiotics are actually tested in order to determine the best combination of effective molecules against the *P. aeruginosa* biofilm and to define optimal conditions for further *in vivo* investigations in a murine model of chronic lung infection.

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P36

An investigation of the role of *Burkholderia cenocepacia* lipopolysaccharide (LPS) O-antigen in modulating the host pro-inflammatory response

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Bacterial species within the CF microbiome demonstrate adaptive strategies including altered expression of LPS. The structure of LPS governs its immuno-stimulatory potential. It is widely accepted that modifications of the lipid A moiety of sequential CF lung isolates can significantly alter the host pro-inflammatory response however similar studies with respect to *B. cenocepacia* O-antigen loss are not as prevalent. It is thus important to elucidate how O-antigen modification impacts the host response. We are investigating LPS extracted from four *B. cenocepacia* sequential isolates cultured from a CF patient during a 3.5 year infection to determine 1) if *B. cenocepacia* LPS is structurally modified within the CF lung, 2) the effect of any modification on the host response to LPS and 3) if LPS from a *B. cenocepacia* mutant strain lacking O-antigen differentially induces the host response when compared to wild type LPS.

We demonstrate as part of this study that the O-antigen domain was present in the LPS from the first *B. cenocepacia* sequential isolate but absent from the three later isolates. Other studies have demonstrated O-antigen loss in *P. aeruginosa* sequential isolates therefore this strategy may be utilised by Gram-negative CF pathogens to facilitate chronic persistence. We also demonstrate that LPS from the later isolates induced a significantly greater pro-inflammatory response from CFBE410- cells ($P < 0.001$), 16HBE140- cells ($P < 0.05$) and HL60 cells ($P < 0.001$) when compared to LPS from the initial isolate. These data suggest that the loss of O-antigen in the later isolates may be responsible for the increased pro-inflammatory response. To verify this, we have successfully deleted the BCAL2405 gene from *B. cenocepacia* K56-2. BCAL2405 is located on chromosome 1 of the genome and encodes an O-antigen ligase that joins the O-antigen domain to the core oligosaccharide domain of the LPS molecule. Deleting the BCAL2405 gene generated a mutant K56-2 strain that lacked LPS O-antigen. We are currently investigating the pro-inflammatory potential of the mutant strain.

P38

Elucidation of the role of peptidoglycan-associated lipoprotein (BCAL3204) in the virulence of *Burkholderia cepacia* complex

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Burkholderia cepacia complex (Bcc) infection may cause a severe necrotising pneumonia and bacteraemia in immunocompromised individuals, including those with CF. While several virulence factors have been identified, the pathogenesis of Bcc is not fully elucidated. We used immunoproteomics to identify potential virulence factors in two clinically relevant species, *B. cenocepacia* and *B. multivorans*. Two-dimensional blots of Bcc proteins were probed with serum from Bcc colonised CF patients. Twelve *B. cenocepacia* and 14 *B. multivorans* immunogenic proteins were identified using MALDI-ToF MS, of which six proteins were common to both species. An OmpA-family lipoprotein/Peptidoglycan-associated lipoprotein (PAL), encoded by the BCAL3204 gene, was chosen to further investigate its role in Bcc virulence. This protein is highly immunogenic and involved in the pathogenesis of other bacteria and was previously shown by our group to be involved in adhesion to lung epithelial cells.

A BCAL3204 targeted deletion mutant was constructed in *B. cenocepacia* K56-2 and its virulence examined using the *Galleria mellonella* infection model. The virulence was significantly reduced ($p < 0.001$) in comparison to the wild type (WT), confirming its pathogenic role. In addition, when larvae were injected with supernatant from this mutant a higher number died compared to the WT supernatant, suggesting the presence of a higher number of extracellular proteins and a role for this protein in membrane structure. Confocal microscopy showed that compared to the WT, the PAL mutant had a two-fold reduction in adhesion to CF bronchial epithelial (CFBE410-) cells, demonstrating that it is required for epithelial cell attachment. As PAL is needed for the transport of O-antigen subunits of *E. coli* LPS, purified LPS fractions from the WT and PAL mutant were compared but no difference in the structure of the O-antigen was observed. This suggests that decreased virulence and attachment of the mutant was independent of LPS composition.

PAL was recombinantly expressed and purified using an *E. coli* expression system. The adhesion of PAL-expressing recombinant *E. coli* to CFBE410- cells was enhanced compared to WT *E. coli*, confirming that it plays a direct role in attachment to host cells. Recombinant PAL elicited concentration-dependent increases in IL-6 and IL-8 secretion in comparison to control supernatants, indicating PAL is also involved in the inflammatory response in host cells.

Circular dichroism was used to determine PAL structure and thermal gradient analysis showed that the protein is quite stable with a high melting temperature and that thermal unfolding is fully reversible. PAL was shown to bind comparably to peptidoglycan (PGN) from both Gram-positive and Gram-negative bacteria and the PGN binding site has been identified.

Work is ongoing to further characterise the role of PAL in Bcc pathogenesis in order to evaluate its potential as a novel drug target for anti-virulence therapies.

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The host response to iron sequestration by *B. cenocepacia*

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B. cenocepacia is a highly antibiotic resistant, opportunistic pathogen that can cause serious infection in CF patients. Iron acquisition is central to this pathogen's ability to colonise the host. *B. cenocepacia* produce low molecular weight iron chelators termed siderophores that bind iron in the host, the most abundant being ornibactin. To combat this mechanism mammals produce lipocalin proteins, some of which can bind siderophores. This study demonstrated that in iron deplete cultures (0 μ M) and (1 μ M) *B. cenocepacia* siderophore production was significantly upregulated by 4 h ($P < 0.001$) with maximal production at 5 h. At iron concentrations (2 μ M) and (3 μ M) siderophores response was significantly upregulated after 6 h ($P < 0.001$) and at maximal by 7 h. We have also shown *B. cenocepacia* has the ability to utilise exogenous iron sources such as host iron binding proteins and xenosiderophores from the co-colonising species *Aspergillus fumigatus*. These abilities were shown to be independent of siderophores production. Utilising ornibactin deficient mutants, we have demonstrated a 7-fold increase in growth with ferritin (10 μ M) ($P < 0.001$) and a 6-fold increase with hemin (10 μ M) ($P < 0.001$). Similarly, wild-type strains showed a 16 and 10 fold increase in the presence of ferritin (10 μ M) and hemin (10 μ M), respectively. The iron binding proteins lactoferrin and transferrin had no effect on growth. *B. cenocepacia* ornibactin negative mutants and wild type strains both exhibited significantly increased growth in the presence of both ferrated-triacetylfusarinine C (10 μ M) and ferrated-fusarinine C (10 μ M), indicating the potential for co-colonising species competition and symbiosis. To determine the host response to siderophores, lipocalin 1 (LCN1) was recombinantly expressed in *E. coli* using the Champion™ pET 100 vector system. We have demonstrated LCN1 has a high binding affinity for ferric-ornibactin and not unferrated ornibactin. In order to further investigate the host response, the cellular lipocalin response will be determined. Iron acquisition systems facilitate colonisation, understanding these mechanisms along with the host response are necessary in the battle to prevent infection in CF patients.

P40

Neutrophil elastase-cleaved SPLUNC1 disrupts *Burkholderia cepacia* biofilms better than intact SPLUNC1

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Cystic fibrosis is genetic multi-organ disease caused by absent or dysfunctional CFTR mediated Cl⁻ secretion which in the lung, leads to mucus obstruction and chronic infection/inflammation. Short Palate Lung and Nasal Epithelial Clone 1 (SPLUNC1) is an innate defense protein that is secreted by the upper airways. The *Burkholderia cepacia* complex (BCC) is a ubiquitous family of environmental microbes; some of which, for example, rot onions. Whilst generally harmless to humans, they are extremely pathogenic to CF patients and acquisition of BCC is associated with a significant increase in mortality. Whilst SPLUNC1 is known to have anti-microbial functions, its effects on the BCC are not known. Chronic neutrophilia is a hallmark of CF lung disease and SPLUNC1 function has been shown to be impaired following SPLUNC1 degradation with neutrophil elastase. We therefore tested the hypotheses that (i) SPLUNC1 was able to impair BCC biofilm formation and (ii) that neutrophil elastase impaired SPLUNC1's anti-biofilm actions. BCC clinical isolates from CF patients (J2315 and AU1054) were cultured as biofilms for 24 h under standard conditions. SPLUNC1 was then added over the range 0.1 – 100 µg/ml and biofilm growth measured. For J2315, ≥10 µg/ml SPLUNC1 disrupted biofilm formation. For AU1054, ≥1 µg/ml SPLUNC1 disrupted biofilm formation. We have recently elucidated the crystal structure of SPLUNC1. We therefore tested novel mutants based on this structure. D44 SPLUNC1, which lacks the S18 region, Δalpha4 and Δalpha6 which lack novel alpha helices all disrupted J2315 biofilm to a similar extent as WT SPLUNC1 (all n=3; p>0.05). In contrast, S18 peptide had no effect on J2315 biofilm growth (n=3; p>0.05). To cleave SPLUNC1, we exposed 1 µM SPLUNC1 to 100 nM NE for 1 h, after which time, we halted NE activity by adding the NE inhibitor ONO5046. Degradation of SPLUNC1 was confirmed by SDS-PAGE. NE ± ONO5046 had no effect on biofilm growth. Surprisingly however, the effect of SPLUNC1 to disrupt biofilm formation was significantly potentiated by NE pretreatment (n=3; p< 0.001). Whilst SPLUNC1's mechanism of action is not known, it appears that NE enhances SPLUNC1's ability to disrupt biofilms, suggesting that different components of the lung's innate immune system may work synergistically. Whilst the impact of CF on SPLUNC1-BCC biofilm interactions is not currently known, or data suggest that understanding this interaction may have important implications for CF lung disease.

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P41

Development of new inhibitors of the non-lysosomal β -glucosylceramidase GBA2 as possible anti-inflammatory agents for CF lung disease

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Cystic fibrosis (CF) lung disease is characterized by progressive chronic infection and inflammation of the airways. Resultant progressive airways remodeling leads to irreversible damage and fibrosis, which are a major cause of mortality in patients.

Current anti-inflammatory strategies for the treatment of pulmonary disease in CF are limited; thus, there is continued interest in identifying additional molecular targets for therapeutic intervention. Interestingly, several lines of evidence highlight the involvement of ceramide accumulation in various respiratory disorders, including CF. In particular, we found that the infection of bronchial epithelial cells with *Pseudomonas aeruginosa* (PAO1 strain) results in the increase of the cell surface ceramide content. On the other hand, we demonstrated that the non-lysosomal β -glucosylceramidase GBA2 inhibition by both miglustat and *N*-(5-adamantane-1-yl-methoxy)pentyl)-deoxynojirimycin (Genz-529648) results in a reduction in the formation of ceramide after PAO1 infection. Moreover, in these conditions we also observed a strong anti-inflammatory effect as indicated by the reduction of IL-8 mRNA levels and protein release upon the PAO1 infection (Loberto, 2014). Compared to miglustat, the treatment of CF bronchial cells with the iminosugar Genz-529648 causes a very similar maximal inhibition of 50% in the inflammatory response to the PAO1 infection. Interestingly IC₅₀ value (2-9 nM) was considerably lower than that obtained with miglustat (2 μ M). Since the modification of the lead iminosugar deoxynojirimycin DNJ with the adamantane moiety (AMP) results more efficient, both in term of GBA2 inhibition and anti-inflammatory response, a small library of neoglycoconjugates containing AMP has been synthesized (Guisot, 2011). Interestingly, by varying the length of the alkyl chain linking DNJ and AMP, we found that shorter conjugates were similarly effective in reducing the inflammatory response to *P.aeruginosa* in CF bronchial cells, being the IC₅₀ values obtained with each compound very similar (0.4-1 μ M). These results demonstrate that AMP moiety improves the potency of inhibition, thus leading to increase the selectivity for the molecular target. The length of the alkyl chain between the iminosugar and AMP seems to be crucial, since potency of shorter compounds is lower than that observed with Genz-529648. Our findings further support the use of modulators of the metabolism of the ceramide for CF lung inflammation and provide evidence that these iminosugars are effective even at low dosages, thus limiting potential adverse effect.

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P42

Passage-dependent effects of CF bronchial epithelial cells

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Background: Advanced age is associated with increased susceptibility to infection through changes in lung physiology and immune function ("immunosenescence") and an increased activation of pro-inflammatory transcription factor NF- κ B has been demonstrated in various tissues in the elderly. ***Advances in CF treatment will see an 'ageing CF population' in the future but the effect of age on an already compromised 'CF immune system' is unknown.***

The aim of this study was to investigate passage-dependent effects on 16HBE14o- and CFBE41o- cell lines at baseline and following pro-inflammatory stimulation, with respect to cellular proliferation, cytokine release and expression of NF- κ B(p65).

Methods: 16HBE16o- and CFBE41o- were cultured in collagen coated flasks to reach the following passage number: 'Young' cells: passage 6-15; 'Old' cells: passage 38-42. For stimulating with LPS (*P aeruginosa*, SIGMA, 10 mg/ml) cells were seeded into 24 well plates at 10^5 /ml and stimulated for 0-24h. We determined cytokine release (IL-6, IL-8, ELISA), mitochondrial activity (MTT assay) and NF- κ B(p65) mRNA expression (qPCR). All experiments n=3-5.

Results: In 16HBE14o- , mitochondrial activity significantly decreased with increasing passage number ($p < 0.05$), while 'older' CFBE41o- cells showed significantly higher mitochondrial activity than younger cells ($p < 0.001$). In both cell lines there was no difference in the response to LPS stimulation compared to the age matching non-stimulated cells.

In response to LPS IL-8 release of 'young' cells (16HBE14o- and CFBE41o-) was higher than that of 'older' cells, especially in CFBE41o- (< 0.001). However, in both cells basal and LPS-induced release of IL-6 was significantly higher in 'older' cells (all $p < 0.001$).

In 'young' 16HBE14o- p65 mRNA increased significantly 8h after LPS stimulation ($p < 0.01$), but there was no peak induction in LPS stimulated 'old' 16HBE14o-. CFBE41o- did not show a significant difference in LPS induced p65 induction in neither 'young' nor 'old' cells, but overall CFBE41o- showed higher levels of p65 mRNA expression than 16HBE14o-, in 'young' and 'older' cells.

Conclusion: 16HBE14o- and CFBE41o- cells with increasing passage numbers (40+) show distinct differences in their inflammatory response compared to cells with lower passage numbers (< 20). Additional work is under way to further validate this cell culture model of the ageing airway epithelium. Ultimately, confirmation in primary cells from patients ('young' vs 'older') will be needed.

P43

Investigating the supramolecular organisation of respiratory mucins

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The respiratory tract is protected from pathogenic and environmental challenges through the action of a dynamic and mobile mucus barrier. In cystic fibrosis (CF) a change in the hydration and ionic content of this barrier contribute to aberrant mucus transport properties, which can result in the adherence of mucus plaques to the airway epithelium, thus providing an ideal environment for bacterial growth. Subsequent chronic inflammation and mucus accumulation are key aspects of CF associated morbidity and mortality.

Polymeric mucins are important contributors to the functional and structural characteristics of mucus gels. Arranged in a network-like formation, these large glycoproteins confer viscoelastic properties onto mucosal secretions. The mechanisms behind mucin packaging into secretory granules and expansion after secretion are not completely understood, but may be reliant upon calcium mediated changes to mucin supramolecular topology. In the low pH environment of the secretory granule the binding of calcium to mucin N-terminal protein domains is thought to promote mucin condensation via the formation of non-covalently crosslinked polymers. Using Transmission Electron Microscopy (TEM) and sedimentation analysis we investigated the structural organisation of the airway mucin MUC5B. We demonstrate that respiratory mucins form highly entangled linear polymers, the structures of which are sensitive to Ca^{2+} concentration and fluctuations in pH. In the presence of calcium MUC5B formed highly compact structures which displayed dramatic chain entanglements; however after the removal of calcium chains appeared extended and rigid. Moreover, we provide evidence to suggest that MUC5B contains bead-like structures which repeat along the polymer axis and suggest that MUC5B may consist of folded repeating units rather than the disorganised random coil organisation previously assumed.

P44

The P2X₄ receptor is upregulated in human trachea cells with chronic IL-13 treatment and influences mucin secretion

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In Cystic Fibrosis patients and in other with chronic lung diseases, goblet cell hyperplasia and increased MUC5AC secretion relate to an increase in the number of exacerbations of cough and infection. As we recently reported a “fusion-activated” Ca²⁺-entry (FACE) via vesicular P2X₄ receptors in primary alveolar type II cells (ATII) and that FACE regulates fusion pore expansion and facilitates pulmonary surfactant release during the post-fusion stage of lamellar body (LB) exocytosis (PNAS, 2011 Aug 30; 108(35): 14503-8), we proposed that this model may be extended to goblet cells in the human large airways as well. Goblet cells in the airways are packed with mucin containing vesicles and these vesicles share similar properties with lamellar bodies; thus suggesting that mucin granule exocytosis may be similar to surfactant secretion from ATII cells. The aim of this study was to further investigate the potential role of P2X₄ receptors in mucin secretion under both normal physiological and pathophysiological conditions.

Primary human trachea epithelial cells (HTECs) were grown on Transwell filters at air liquid interface (ALI) for more than 21 days in either the presence or absence of Interleukin-13 (IL-13). IL-13 is well known to induce goblet cell hyperplasia and a hypersecretory phenotype, similar to patients with chronic lung diseases. RT-PCR, immunostaining and western blot experiments confirmed up-regulation of MUC5AC – in the HTEC cells following IL-13 treatment, as previously published. The P2X₄ receptor was observed in control cells and interestingly, was up-regulated in HTECs treated with IL-13. To better understand the function of P2X₄ in mucin secretion; an ELISA assay was performed to study MUC5AC secretion. Surprisingly, stimulation of mucin secretion in the presence of ivermectin – a potent P2X₄ potentiator – resulted in an increased rate of MUC5AC secretion with goblet cell hyperplasia due to chronic inflammation.

Our results indicate that HTECs treated with the inflammatory cytokine IL-13 demonstrate up-regulation of MUC5AC and P2X₄ receptor expression and correlates to a stimulatory effect of P2X₄ receptor activation on mucin secretion. Therefore; the P2X₄ receptor is a promising target for modulating mucin secretion in cystic fibrosis patients where excess mucus is highly problematic.

P45

Imbalance of mucus homeostasis in chronic pancreatitis

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Introduction: Qualitative and/or quantitative changes of mucus in the pancreas can be an important factor both in the initiation and progression of chronic pancreatitis (CP)¹. Imbalance in mucus homeostasis can lead to elevation of fluid viscosity in the ductal lumen which can result slower secretion or mucoprotein plug formation within the pancreatic ducts². Therefore, our main objectives were to (i) investigate the mucus content both in human CP and experimental pancreatitis models and (ii) to correlate the mucus changes with ductal fluid secretion and pancreatic fibrosis.

Methods: Human and mouse pancreata were investigated. Human CP tissue samples were collected from surgically resected pancreata, whereas CP was induced by administration of 6x50µg/kg cerulein 3 times a week for 4 weeks period in mice. Morphometric analysis of mucus was carried out by CellF software. Total RNA was isolated from human and mouse tissue. The mRNA levels of different mucin subtypes were analysed by quantitative RT-PCR. Fluid secretion into the closed luminal space of the cultured pancreatic ducts was analysed using a swelling method, whereas the level of fibrosis was determined by trichrome staining.

Results: The mucus volume density ($V_{d_{muc}}$) of human PDEC was significantly higher in CP than in controls, in case of smaller ducts (ductal diameter < 100µm: $1.21 \pm 0.13 \text{ nl/mm}^2$ and $0.37 \pm 0.05 \text{ nl/mm}^2$, respectively). Similarly, mouse PDEC showed significantly higher $V_{d_{muc}}$ in CP than in controls, especially in ducts with smaller diameter (ductal diameter < 80µm: $0.72 \pm 0.06 \text{ nl/mm}^2$ vs. $0.005 \pm 0.0002 \text{ nl/mm}^2$, ductal diameter > 80µm: $0.075 \pm 0.020 \text{ nl/mm}^2$ vs. $0.016 \pm 0.004 \text{ nl/mm}^2$). Mucin gene expression analysis showed, that muc6 was ~1000-fold upregulated in mouse and 17-fold upregulated in human CP. Changes in the relative luminal volume of isolated pancreatic ducts from cerulein treated mice show an abolished basal and stimulated fluid secretion after 4 weeks of treatment. Analyses of the time course changes of mucin, fibrosis and ductal secretion during the first four weeks showed clear association between the elevation of mucus content, decrease in fluid secretion and accretion of fibrosis.

Conclusion: There is a clear quantitative and qualitative changes in mucus homeostasis during the development of CP. Our data suggest that these changes are in association with decreased fluid secretion and increased fibroses, however, the cause-and-effect relationship needs to be determined. This study was supported by MTA, OTKA, TAMOP and EPC fellowship.

1. Sarles, H. *et al.* Observations on 205 confirmed cases of acute pancreatitis, recurring pancreatitis, and chronic pancreatitis. *Gut* (1965).
2. Hegyi, P. & Petersen, O. H. The exocrine pancreas: the acinar-ductal tango in physiology and pathophysiology. *Rev. Physiol. Biochem. Pharmacol.* (2013).

P46

Structure activity relationship for OligoG-induced normalization of the CF mucus phenotype

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Objectives: Cystic fibrosis (CF) is a recessive genetic disease caused by nonfunctional chloride and bicarbonate ion transport via CFTR. In the lungs of these patients, dense, intractable mucus collects because it is insufficiently cleared by the mucociliary clearance system, leading to bacterial colonization, lung infections and tissue damage.

We have previously shown that the gel-forming MUC2 mucin is packed in an orderly way that allows unpacking by calcium chelation and increased pH (1). Using an explant system (2), we found that, in contrast to normal mucus, the mucus of the small intestine is attached to the epithelium in mice without a functional CFTR channel (CfrF508-del). This mucus could be reverted to a non-attached phenotype by apical solutions containing above 100 mM bicarbonate (3).

OligoG CF-5/20 (AlgiPharma, Sandvika, Norway) is a refined oligosaccharide mixture derived from polymeric sodium alginate comprising mainly guluronate and some mannuronate residues, with average length of 13 monomers. OligoG is currently in clinical trials as inhalation therapy in CF patients. We have now tested and compared OligoG and alginate subfractions with different composition on mouse ileum CF explants and recorded to what extent OligoG can affect the attached CF mucus.

Methods: Explants from the small intestine of Cfr508-del mutant mice were mounted in the horizontal Ussing-type chamber (2). Alginate at different concentrations or composition was added to the apical buffer, pH 7.4. The attachment of the already formed mucus was assessed by comparing the total mucus thickness before and after aspiration.

Results: OligoG at 1.5% or higher normalized the mucus phenotype without increase in mucus thickness. At 1% OligoG the mucus remained attached and at 1.2% an intermediate phenotype was observed. OligoG fractions containing an average of 12 guluronate units were most effective, whereas shorter and longer molecules were less efficient. Mannuronic polymers were inactive in this assay.

Conclusion: These results suggest that it is guluronic acid and its calcium chelating capacity that is the important and active component in OligoG. These observations indicate that OligoG could act by normalizing the mucus layers in both the intestine and potentially the lungs of cystic fibrosis patients at therapeutically relevant concentrations.

1. Ambort, D., Johansson, M. E. V., Gustafsson, J. K., Nilsson, H., Ermund, A., Johansson, B. R., Kock, P., Hebert, H., & Hansson, G. C. (2012) *Proc. Natl. Acad. Sci. U. S. A.***109**, 5645-5650.
2. Gustafsson, J. K., Ermund, A., Johansson, M. E. V., Schutte, A., Hansson, G. C., & Sjovall, H. (2012) *Am. J Physiol Gastrointest Liver Physiol.***302**, G430-G438.
3. Gustafsson, J. K., Ermund, A., Ambort, D., Johansson, M. E. V., Nilsson, H. E., Thorell, K., Hebert, H., Sjovall, H., & Hansson, G. C. (2012) *J. Exp. Med.***209**, 1263-1272.

P47

Mechanism-based screens lead to the identification of complementary pharmacological correctors of F508del CFTR

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The most prevalent form of Cystic Fibrosis is caused by F508del in CFTR. This mutation leads to misfolding of the nucleotide-binding domain 1 (NBD1) and perturbs normal interdomain interactions in the CFTR protein, resulting in the absence of functional CFTR chloride channels in the plasma membrane. Recent progress suggests targeting multiple steps in CFTR folding (e.g., NBD1 folding and interdomain interaction) may result in much more efficacious correction of CFTR F508del. However, the first generation of F508del correctors, which was discovered through phenotypic screening, appears to largely address just one aspect of the complex misfolding.

Harnessing the genetic clustering of suppressor mutations of F508del, we developed allelic screens with the goal to enrich for small molecules that preferentially modulate NBD1 folding or the interdomain interactions. A big proportion of F508del partial suppressors can be classified as NBD1 stabilizers or interdomain modifiers. Mutations from these two classes can complement to produce significantly more correction of F508del. The screening relies on CFBE410⁻ cells expressing CFTR F508del with partial suppressors of either NBD1 misfolding (3S) or interdomain perturbations (R1070W), and horseradish peroxidase (HRP) reporter is inserted into an extracellular loop of CFTR. Such a reporter allows a robust read-out for CFTR in the plasma membrane. Screening of more than 70,000 compounds at 10 μ M in both CFTR F508del 3S and F508del R1070W alleles resulted in different classes of hits. To profile these hits a mechanism-based screening index was developed in addition to the efficacy indices in both cell lines. The hits (>40% over vehicle) are characterized not only for the efficacy of F508del correction but also for the specific enrichment in either allele. The rates of both hit confirmation and translation into CFTR function (electrophysiology) and trafficking (Western analysis) are greater than 67%. Of these hits, about 15% have demonstrated corrector activity in primary hBE cells. Several hits could be expanded to early series with F508del corrector activity in primary CF hBE cells. The most advanced series to date reaches the efficacy level of VX-809 before any chemical optimization.

To target multiple steps in CFTR folding, corrector combination screening has been developed using randomized pairs of two correctors for which we measure the efficacy of rescuing the disease allele CFTR F508del. A single concentration was selected for each corrector based on its apparent EC₉₅ in primary CF hBE cells. A confirmation assay has also been developed in the form of dose response matrix of the corrector pair. The limited combination screening leads to the discovery of complementary corrector pairs that achieve significantly greater levels of F508del correction than VX-809 alone. Our approach is neither dependent on nor limited to any existing corrector. Hence it can be applied to any pharmacological development of F508del correction. Further application of the corrector combination screening will help to select compounds based on their potential for pharmacological complementation.

P48

Predicting the individual clinical efficacy of CFTR-modulating drugs using rectal cystic fibrosis organoids

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The identification of subjects with cystic fibrosis (CF) that can benefit from CFTR-modifying drugs is time-consuming and costly, and especially challenging for individuals with rare, non-characterized *CFTR* mutations. Here, we studied individual CFTR function and response to the prototypical CFTR potentiator VX-770 and corrector VX-809 in rectal organoids derived from 69 individuals expressing wild-type *CFTR* or various class I-V *CFTR* mutations. We observed that CFTR residual function and response to therapy are continuous and not categorical variables that depend on both the *CFTR* mutations and the individual's genetic background. Most importantly, in vitro drug responses assessed in organoids positively correlated with published outcome data of clinical trials with VX-770 and VX-809, allowing us to preclinically identify potential in vivo responders with rare *CFTR* mutations. We demonstrated proof-of-concept that subjects with extremely rare *CFTR* mutations can successfully be selected for therapy using functional organoid measurements by identifying a subject expressing a non-characterized *CFTR* genotype whose in vivo CFTR-dependent biomarkers normalized and clinical parameters improved upon VX-770 treatment. These data strongly indicate that in vitro CFTR function measurements in rectal organoids can play an important role in identifying subjects that can benefit from CFTR-targeting treatment, independent of the *CFTR* genotype.

P49

Measurements of functional responses in human primary bronchial and nasal cells as a basis for personalized therapy for cystic fibrosis

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Background: The best investigational drug to treat Cystic Fibrosis (CF) patients with the most common CF-causing mutation (F508del) is VX-809 (Lumacaftor) which recently succeeded in Phase III clinical trial in combination with Ivacaftor. This corrector rescues F508del-CFTR from its abnormal intracellular localization to the cell surface, a traffic defect shared by all Class II CFTR mutants.

Aim: Our goal here is to test the efficacy of Lumacaftor in other Class II mutants in primary human bronchial epithelial (HBE) cells derived from CF patients and to establish whether human nasal epithelial cells (HNE) can be used for the same purpose.

Methods: The effect of Lumacaftor was investigated in primary HBE cells from non-CF and CF patients with F508del/F508del, A561E/A561E, N1303K/G542X, F508del/G542X and F508del/Y1092X genotypes by measurements of Forskolin plus Genistein-inducible equivalent short-circuit current ($I_{eq-SC-Fsk+Gen}$) in perfused open-circuit Ussing chambers. Efficacy of corrector C18 was also assessed on A561E/A561E and F508del/F508del cells. HNE cellswere cultured using Rho kinase inhibitor (Y-27632) in combination witha feeder layer of fibroblasts [1,2].

Results: Our data indicate that A561E (when present in both alleles) responds positively to Lumacaftor treatment at equivalent efficacy of F508del in primary HBE cells. Similarly, Lumacaftor has a positive impact on Y1092X, but not on N1303K. Our data also show that cells with only one copy of F508del-CFTR respond less to VX-809. Moreover, there is great variability in Lumacaftor responses among F508del-homozygous cells from different donors. Compound C18 failed to rescue A561E-CFTR but not in F508del-CFTR, thus plausibly it has a different mechanism of action distinct from Lumacaftor. Monolayers of HNE cells from non-CF individuals have CFTR-mediated currents ($I_{eq-SC-Fsk+Gen}$) of 21.96 ± 0.17 and ATP-inducible currents ($I_{eq-SC-ATP}$) of 21.2 ± 2.87 . Analysis of HNE cells from CF patients and their responses to compounds of therapeutic potential is ongoing.

Conclusions: CF patients with A561E (and likely also those with Y1029X) can potentially benefit from Lumacaftor. Moreover, the methodology used here exemplifies how *ex vivo* approaches using nasal epithelial models have a good potential to test CFTR and CaCC therapies for CF in a personalized medicine approach.

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[1] Suprynawiczet al (2012) *PNAS USA***109**: 20035–40.

[2] Liu et al (2012) *Am J Pathol***180**: 599–607.

P50

Rescuing F508del-CFTR in airway epithelial cells using a novel traffic-based high content microscopy screening platform

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The F508del-CFTR mutation – primary cause of 85% Cystic Fibrosis (CF) cases – generates a misfolded protein which does not accumulate in the plasma membrane (PM) of epithelial cells and exhibits deficient chloride (Cl⁻) transport activity. Instead, it is retained in the endoplasmic reticulum and prematurely targeted for degradation by the ER quality control (ERQC), rendering CF a protein traffic disorder. Lungs and airways are among the most severely affected organs. Even though the central role of CFTR in CF has been known for over 25 years, a disease curing therapy only exists for about 5% of all patients through Ivacaftor (VX-770). Rescuing F508del-CFTR has proven more challenging because of the multiple defects in the molecule[1]. Nevertheless, F508del-CFTR is capable of residual Cl⁻ transport activity if persuaded to traffic to the PM[2]. Yet, current drug development platforms do not rely on CFTR traffic as a primary readout, thereby potentially justifying the limited success in the development of CFTR-targeting therapeutic drugs, with the notable exceptions of Ivacaftor[3] and Lumacaftor (VX-809)[4].

Our aim is to identify novel genes which rescue F508del-CFTR traffic to the PM as potential therapeutic targets for CF.

To this end an mCherry-CFTR traffic reporter with an extracellular Flag-tag has been stably expressed in respiratory epithelial cell lines – A549 or CFBE – under the control of an inducible (Tet-ON) promoter[5]. These cell lines were used to perform systematic siRNA knockdowns coupled to an immunofluorescence assay to detect PM-located CFTR in high-throughput microscopy mode. Overall, the cell lines and biological assay constitute a novel high-throughput screening (HTS) system for target gene identification and drug development for CF. The robustness of the HTS system was validated and demonstrated by using the 384-well plate format, through which up to 2,000 siRNAs can be tested per week with about 500 cells being analysed per well, *i.e.* per siRNA treatment.

Screening one third of a druggable genome siRNA library (*i.e.*, 3751 siRNAs targeting 2397 genes, Ambion) revealed 178 siRNAs enhancing and 126 siRNAs inhibiting wt-CFTR traffic in A549 cells. Secondary (validation) screens performed in A549 and CFBE cells confirmed hits including COPI coat components, G protein-coupled receptors, chaperones and proteins involved in sterol binding, β -catenin signalling, nuclear/chromatin structuring, cytoskeletal dynamics, cell differentiation and channel regulation. Moreover, preliminary experiments identified several siRNAs rescuing F508del-CFTR to the PM, currently undergoing validation. The relevance of the hits so far identified will be highlighted in the context innovative CF therapeutics and basic cell biology.

In conclusion, the applicability of the system to screen siRNAs/chemical compounds has been validated and the hits so far identified are promising targets for drug development.

1. Farinha et al (2013) Chem Biol 20:943-55
2. Pasyk & Foskett (1995) JBC 270:12347-50
3. Accurso et al (2010) NEJM 363:1991-2003
4. Van Goor et al (2011) PNAS 108:18843-8
5. Almaça et al (2011) Methods Mol Biol 742:249-64

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P51

Anchoring to the actin-cytoskeleton is not essential to retain functional F508del-CFTR at the plasma membrane

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The most frequent mutation causing Cystic Fibrosis (CF) is the deletion of a phenylalanine residue at position 508 (F508del) in the CFTR (CF transmembrane conductance regulator) gene. CFTR is a chloride channel, normally expressed at the surface of epithelial cells, and the F508del mutation causes the protein to misfold and be prematurely degraded. However, if allowed to reach the cell surface, the defective protein would retain a degree of functionality that could lessen the disease severity.

Pharmacological correctors are now available that partially rescue F508del-CFTR trafficking to the plasma membrane (PM). Nevertheless, the rescued channels exhibit a decreased half-life at the PM, decreasing the effectiveness of the drugs.

We previously demonstrated that the retention of rescued F508del-CFTR at the cell surface is required to achieve the best restoration of CFTR-mediated ion transport by the available pharmacological correctors, namely VX-809 (Lumacaftor). We have also observed that activation of Ezrin via PIP5K-mediated production of PIP2 is a key step for the retention of rescued F508del-CFTR at the PM, through its anchoring to the actin cytoskeleton via NHERF1 and ezrin.

To determine if the physical binding to the actin cytoskeleton was the only determinant of F508del-CFTR retention at the cell surface, we used an Ezrin mutant lacking the actin-binding domain (Ezrin- Δ Act), but still capable of binding CFTR/NHERF1 complexes, and assessed the effects of its expression in the rescue of F508del-CFTR activity by VX-809.

Using halide-sensitive YFP (HS-YFP) functional assay, we observed that in VX-809 treated cells F508del-CFTR function was stimulated up to nearly 7-fold by Ezrin- Δ Act expression. Moreover we show that stimulation is directly proportional to the level of Ezrin- Δ Act expressed and completely abolished by co-incubation with CFTR inhibitor 172. This indicates that F508del-CFTR physical anchoring to the actin cytoskeleton is not mandatory for its functional rescue and that additional unknown events may be involved in the retention of CFTR at the cell surface.

These novel insights into the cell surface stabilization of CFTR provide new attractive topic for CF research, aiming the improvement of currently available therapeutic strategies.

P52

Cholapods mediate anion transport across epithelial cell membranes

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Cholapods are steroid-based synthetic anion transporters derived from cholic acid that bind anions with high affinity and promote their efflux from liposomes. To understand better cholapod-mediated anion transport, we studied the cholapods AS09, LJ09 and TL145 in planar lipid bilayers, single cells and polarised epithelia. When compared with AS09, LJ09 had higher anion affinity, whereas TL145 had greater lipid solubility. In planar lipid bilayers, cholapod-mediated currents relaxed to a steady-state after an initial peak when membrane voltage was stepped. Anion transport by cholapods was concentration-dependent with anion conductance decreasing with increasing anionic radius. Evaluation of the rate constants for anion transport by cholapods using the method of Läuger (Science 1972;178:24-30) suggested that movement of the anion-transporter complex across the membrane is the rate-limiting step of cholapod-mediated anion transport. To investigate cholapod mediated anion transport in single cells, we used Fischer rat thyroid epithelial cells stably expressing the halide-sensitive yellow fluorescent protein (FRT-YFP) (gift from A Verkman, UCSF) to monitor anion influx. In the absence of cholapod, addition of iodide (10 mM) to FRT-YFP cells had little or no effect on fluorescence. However, in the presence of cholapod, YFP fluorescence decreased following iodide influx into FRT-YFP cells and when iodide was washed from the external solution fluorescence quenching reversed. Finally, to evaluate cholapod-mediated transport at the tissue level, we grew FRT-YFP cells as polarised epithelia and measured transepithelial Cl⁻ movement in Ussing chambers. Treatment of FRT-YFP epithelia with cholapods led to the development of transepithelial Cl⁻ current that was absent in untreated epithelia. Because TL145 was the most effective transporter in all assays, we conclude that lipid solubility is an important determinant of transmembrane anion transport by the cholapod family of synthetic anion transporters.

P53

Identification of novel potentiator compounds that do not decrease membrane density of $\Delta F508$ -CFTR

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Cystic fibrosis (CF) is a debilitating disease caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene, which codes for the CFTR anion channel.

YFP H148Q/I152L is a GFP derivative, whose fluorescence is quenched by bound anions, with I⁻ binding ~100-fold more tightly than Cl⁻. When coexpressed with CFTR it allows monitoring of CFTR-mediated anion flow and therefore screening of compound libraries (Galletta et al. 2001. *Am. J. Physiol. Cell. Physiol.* 281:C1734-C1742). Here we present a YFP-CFTR fusion protein, in which YFP is tagged to the N-terminal of the CFTR coding sequence. In the presence of high extracellular [I⁻], and following activation by forskolin, open CFTR channels provide a pathway for I⁻ influx which results in an immediate, transient quenching of the corresponding YFP chromophore. Thus YFP-CFTR quenching provides a readout more closely reflecting I⁻ flux through open CFTR channels than quenching of co-expressed, soluble YFP.

HEK293 cells expressing YFP- $\Delta F508$ -CFTR and incubated at 30°C, were used to screen a small library of compounds with some structural similarity to known potentiator VX-770. 3 compounds were identified as novel potentiators, with efficacy on $\Delta F508$ -CFTR of $67 \pm 8\%$, $67 \pm 6\%$ and $52 \pm 6\%$ (n=6) compared with VX-770. These potentiated G551D-CFTR with greater efficacy than $\Delta F508$ -CFTR, as occurs with VX-770, confirming that the signal was mediated by CFTR.

Recently published studies suggest that chronic treatment with VX-770 decreases $\Delta F508$ -CFTR density at the plasma membrane, potentially limiting the clinical effectiveness of combined corrector and VX-770 treatment (Veit et al. and Cholon et al. 2014. *Sci. Transl. Med.* 246(6)). Therefore, we tested the effect of chronic treatment with VX-770 or our compounds on YFP- $\Delta F508$ -CFTR quenching, as a measure of membrane density. Preliminary results confirm that chronic VX-770 treatment causes a dose-dependent reduction in YFP- $\Delta F508$ -CFTR quenching. However, no decrease in quenching was detected following chronic treatment with any of the 3 identified compounds.

This work describes the optimisation of an assay to screen for $\Delta F508$ -CFTR potentiators, and its use to identify 3 VX-770-related potentiator compounds. The 3 novel compounds show promise in that they do not appear to destabilise $\Delta F508$ -CFTR following chronic treatment. Single channel patch clamp experiments to elucidate the compound mechanism of action are ongoing.

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P54

Identification of CF mutations in non-coding regions: design of antisense oligonucleotides for a targeted therapeutic approach

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Objectives: We previously developed an approach for a complete resequencing of the *CFTR* locus*. By using two different benchtop sequencers, we searched for unidentified mutations, deeply located in introns. As model, we focused on CF patients with only one disease-causing mutation. After identifying candidate intronic mutations and determining their functional impact, the aim of this work is to restore full-length *CFTR* transcripts by using antisense oligonucleotides also named Target Site Blockers (TSB).

Materials and methods: Fifteen DNAs with only one CF mutation were studied (the latter not fully genotyped CF DNAs of France). A pre-amplification of the whole *CFTR* locus (274 kb) by Long Range PCR was used. Sequencing was then performed using a 454 GS Junior and a MiSeq Illumina platform. We also developed an in-house pipeline to easily find new mutations in introns. *In silico* and functional analyses were carried out to confirm the deleterious impact of identified variants.

Results: Using our in-house pipeline, among the 200 variations detected (in average per DNA), 10 variations were deeply located in introns and were further studied. Out of 15 DNAs sequenced, 13 putative second mutations were predicted by *in silico* analysis tools to affect *CFTR* splicing by insertion of a cryptic exon. When predictions are confirmed by *ex vivo* functional studies, TSB (Exiqon) are specifically designed to block the creation of pseudoexons. For instance, tests have been carried out for the fourth most frequent mutation in Southwestern Europe, the c.1680-886A>G, and a new identified c.1680-883A>G. TSB transfection resulted in the restoration of the full-length, in-frame *CFTR* transcript in bronchial cells and primary nasal cells. Using minigene constructions, this corrective effect was maintained 48 hours after transfection at low concentration (50 nM). Using this approach of TSB, two new mutations in introns 18 and 23 are being tested.

Conclusion: By using DNA resequencing and our in-house pipeline, we correctly identified CF-causing mutations in introns. Functional analysis confirmed that several deep intronic mutations lead to inclusion of pseudoexons. To date, the efficiency of oligonucleotides as TSB has been validated for two deeply located mutations. These data demonstrate the importance of antisense oligonucleotides strategy for a targeted therapeutic of cystic fibrosis.

This work is supported by Vaincre La Mucoviscidose.

Bonini J *et al., Small-scale high-throughput sequencing-based identification of new therapeutic tools in cystic fibrosis, Genet Med. 2015.

P55

Characterization and correction of CFTR mutations bearing premature termination codons (PTCs)

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Among the ~1,900 CFTR gene variants so far described ~8% are nonsense variations. Moreover, despite the drug discovery efforts to correct F508del, 15% of all patients worldwide lack this mutation in both their CFTR alleles. Nonsense mutations introduce premature termination codons (PTC) usually leading to the loss of the respective transcripts via the mRNA surveillance mechanism termed Nonsense-Mediated mRNA Decay (NMD).

Our aims here are:

- 1) to assess levels of NMD in native tissues of CF patients materials bearing nonsense mutations;
- 2) to study NMD of CFTR transcripts bearing PTC's *in vitro* using a CFTR minigene model;
- 3) to establish a fluorescent assay to be used in microscopy screens for genes modulating NMD and to screen for compounds with read-through activity.

Levels of CFTR transcripts we assessed in native tissues (nasal cells and rectal biopsies) from CF-patients with different mutations generating PTCs. For the *in vitro* studies, stable HEK293 cell lines were generated to express CFTR mini-genes comprising the full CFTR cDNA sequence containing nonsense mutations and the adjacent introns.

Our data show the following percentages of mRNA decay in native tissues: -10±3,2% (Q39X); 80,3±4,4% (G542X); 40,4±0,9% (R553X); 61.6%±1.2% (Q637X); 79.0%±3.7% (2183AA>G); 79.6%±1.7% (Y1092X); 50,2±0,8% (3905insT); 58.2%±1.5% (W1282X). These data indicate that Q39X transcripts avoid NMD, likely due to previously described alternative initiation codons [1].

RT-PCR analysis of transcripts from the *in vitro* mini-genes show that the Q2X, S4X and Q39X- containing CFTR transcript (ex1) escape NMD yielding stable mRNAs, whereas the G542X and Y1092X- containing CFTR transcripts are sent for NMD. Furthermore, by Western Blot the respective N-truncated CFTR proteins were detected. However, G542X (ex11) transcripts induced NMD since no mRNA was detected.

To understand and identify factors involved in NMD as potential drug targets, we are currently producing similar NMD CFTR mini-gene models, containing full-length CFTR cDNA fused to GFP at the C-terminus and containing the three above mutations with different NMD fate: Q39X, G542X and Y1092X, which lead to different levels of CFTR transcripts. This model will allow to use automatic fluorescence microscopy to testing siRNA/ compound libraries for CF drug discovery.

[1] Ramalho *et al* (2009) *Cell Physiol & Biochem* **24**: 335-346.

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P56

***In vitro* correction of the CFTR 2657+5G>A splicing mutation by an antisense oligonucleotide**

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Cystic fibrosis (CF) is caused by mutations in the CF transmembrane conductance regulator (*CFTR*) gene, and approximately 2000 different *CFTR* gene variants have been described most presumably pathogenic. A significant fraction of these (~12%) affects pre-mRNA splicing. The potential pathogenic nature of many non-obvious splicing variants as well as novel therapeutic approaches that correct these defects have been neglected. We have previously described the effect of the *CFTR* intronic splicing mutation c.2657+5G>A (legacy name 2789+5G>A) in IVS16, and showed that it leads to aberrant transcripts lacking exon 16 as well as wt transcripts [1].

Here, we aimed to correct the aberrant splicing caused by the c.2657+5G>A splicing mutation using an RNA-based antisense oligonucleotide (AON) strategy. To this end, two distinct AONs complementary to the pre-mRNA area of interest in IVS16 were designed and tested for their effects in correcting the c.2657+5G>A mutation in a minigene consisting in full-length *CFTR* cDNA with IVS14, IVS15 and IVS16 stably expressed in HEK cells by use of single insertion Flip-in pCDNA5/FTR/*CFTR* vector.

Our data show that the AON1 (but not AON2) significantly restored exon 16 inclusion in *CFTR* mRNA derived from HEK 293 cells stably expressing the above *CFTR* minigene carrying the 2657+5G>A mutation. Consistently, AON1 increased levels of full-length *CFTR* protein. Our ongoing studies investigate levels of *CFTR* at the plasma membrane and function.

In conclusion, our *in vitro* studies revealed that the 2657+5G>A mutation leading to exon 16 skipping can be corrected by an AON, thus suggesting that this one as a promising therapeutic approach for correction of such *CFTR* splicing mutation.

[1] Masvidal, Igreja *et al* (2014) *Eur J Hum Genet***22**: 784-91.

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P57

CRISPR/Cas-9 NHEJ based knock-out of CFTR class I mutations to restore splicing

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Whilst there are now therapeutic options for CF patients with the major class II and III mutations, the the most realistic option at the present for patients with class I mutations where no protein is made, is a gene-based therapeutic approach. Precise genome editing using CRISPR/Cas9 HDR (Homology Directed Repair) to repair class II $\Delta F508$ in cell lines has been well established, but only occurs at low efficiency. In contrast, the use of CRISPR/Cas9 gRNAs to delete short sequences by NHEJ (Non Homologous End Joining) or larger sequences by using two gRNAs to create a deletion of essentially the same size as the distance between their respective DSB sites is highly efficient. Thus, we have identified three class I CF-causing intronic mutations that provoke aberrant splicing due to creation of cryptic donor (3849+10kB C>T and 1811+1.6kB A>G) or cryptic acceptor (3272-26 A>G) splicing sites. Together these mutations affect around 750 individuals, nearly 2% of all CF patients. Our objective is to use these two CRISPR/cas-9 NHEJ based techniques to effectively delete these mutated intronic sequences. Removal of these cryptic splice sites created by these mutations should lead to a correctly spliced transcript that will be translated to a functional CFTR protein. Two pairs of gRNA have been designed to flank the deep intronic 3849+10kB C>T and 1811+1.6kB A>G positions. To target the 3272-26 A>G position, we designed a gRNA close to the 5' acceptor site and an upstream gRNA to create a deletion which should restore the splice site. These gRNAs have been successfully cloned in pSpCas9(BB)-2A-GFP, a vector capable of expressing both gRNA and Cas9. The ability to delete the target positions will be initially optimised in CF cell lines using PCR and direct sequencing. To measure functional correction of CFTR, a primary human nasal epithelial (HNE) air-liquid interface cell model will be used; cells from patients with the three different patients have been sourced with ethical permission.

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Modulating rescue of $\Delta F508$ CFTR in human airway epithelial cultures

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The identification of lumacaftor (VX-809), a small-molecule corrector compound that improves $\Delta F508$ CFTR maturation and trafficking, provided an important advance in cystic fibrosis (CF) therapy. VX-809 treatment resulted in significant correction of $\Delta F508$ CFTR *in vitro*, but did not improve pulmonary function as monotherapy in $\Delta F508/\Delta F508$ patients. A subsequent clinical trial that evaluated a combination therapy of VX-809 and the potentiator, ivacaftor (VX-770), showed statistically significant but modest improvement in FEV₁. The goal of our studies is to explore factors that modulate $\Delta F508$ rescue, which may reveal additional strategies and targets for improving $\Delta F508$ function, and allow for optimization of *in vitro* models for CFTR correction.

Fully differentiated primary human bronchial epithelial (HBE) from CF $\Delta F508/\Delta F508$ patients or CFBE41o- $\Delta F508$ cultures were grown at air-liquid interface, and corrected for 48 hours with 5 μ M VX-809. During correction, cultures were also exposed to various factors that influenced correction of $\Delta F508$ CFTR. Correction was also evaluated in cultures grown in the presence of serum substitutes. Ussing chambers were utilized for electrophysiological measurements of CFTR function after correction by VX-809 and after acute stimulation with 5 μ M VX-770. Biochemical analysis by Western blotting was used to determine the magnitude of mutant CFTR rescue. CFTR mRNA expression was evaluated by quantitative RT-PCR.

The milieu of CF airways is characterized by chronic infection and inflammation. We found that culture filtrate of *Pseudomonas aeruginosa*, which often colonizes CF airways, impaired functional and biochemical correction in the airway epithelial cell line, CFBE41o- $\Delta F508$, an effect which varied between different bacterial strains. The increased inflammatory condition found in CF lungs leads to an increase in the expression of genes involved in lipid/cholesterol biosynthesis, increasing cellular cholesterol levels in CF cells. Therefore, we treated cultures with cholesterol modulators and found that reducing cholesterol augmented biochemical correction. Oxygen availability has been described as an environmental stimulus that regulates CFTR function. In examining CO₂ as a potential modulator, we found that exposing primary cultures to 15% CO₂ at 37°C during VX-809 treatment led to considerably enhanced functional correction of $\Delta F508$ CFTR (up to 50-60% higher than in treatment with 5% CO₂ at 37°C), without change in CFTR mRNA expression or biochemical correction. *In vitro* studies often use culture media that may vary due to different formulations and availability of ingredients. While examining culture media conditions, we found that the presence of serum substitutes remarkably affected culture differentiation and modulated CFTR rescue by VX-809 and acute potentiation by VX-770.

Ultimately, the efficacy of VX-809 rescue of $\Delta F508$ is modulated by various conditions such as exposure to bacterial culture filtrate, cellular cholesterol content, CO₂ levels, and culture media serum supplements. A greater understanding of their mechanism may improve strategies for treating $\Delta F508$ with VX-809 and optimize *in vitro* models for further studies.

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Trafficking in CFTR therapies

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Cystic fibrosis (CF) is caused by loss of function mutations affecting CFTR, an ion channel that regulates ion and fluid homeostasis in epithelial tissues. The most common mutation is F508del-CFTR, which fails to fold, exhibits low channel activity, and is rapidly degraded. Existing CFTR modulators facilitate F508del folding and potentiate its ion-channel activity. However, none of them specifically target its post-maturational trafficking, and even in combination, they provide limited benefit (3% FEV₁) for F508del homozygotes and no benefit for heterozygotes. The Disabled-2 (Dab2) CLASP endocytic adapter protein and the CFTR-Associated Ligand (CAL) PDZ protein contribute to the endocytic uptake of F508del from the apical membrane of epithelial cells, limiting its half life and decreasing the net number of functional channels. Inhibitors of Dab2 and CAL are thus expected to extend the half-life of F508del and to increase net channel numbers at the membrane. Here, we present novel peptide and peptidomimetic inhibitors of CAL, designed to facilitate intracellular delivery. Using a mass-spectrometry-based pull-down assay, we demonstrate unprecedented selectivity for the CAL PDZ domain. We also have engineered a nanomolar peptide reporter for the Dab2 DH domain, and deployed it in a pilot HTS to identify small-molecule inhibitors. CAL and Dab2 inhibitors specifically enhance net ion-channel activity in airway epithelial cells. Furthermore, we show that such inhibitors not only increase channel activity on their own, but also can act additively or synergistically with existing folding correctors and gating potentiators. They thus offer the prospect of improved therapy for patients carrying the most common disease-associated CFTR mutation.

P60

Interactions of cytokeratin 8 with misfolded Phe508del-CFTR and Z-alpha-1 antitrypsin – role in protein trafficking and/or degradation

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Introduction and objective: We have reported that cytokeratin 8 (K8) affects the trafficking of misfolded Phe508del-CFTR from the endoplasmic reticulum (ER) to the plasma membrane by interacting directly with Phe508del-NBD1, and that disruption of this interaction leads to correction of functional Phe508del-CFTR (Colas et al., HMG, 2012, 21:623-34; Odolczyk et al., EMBO Mol Med, 2013, 5:1484-501). We hypothesize now that K8 interacts with other abnormally folded proteins, preventing their correct trafficking and secretion, and contributing to pathogenesis. We test this hypothesis on misfolded alpha-1 antitrypsin (A1AT), the Z mutant of A1AT (E342K). A1AT is an inhibitor of inflammatory proteases that protects the lungs from degradation. Misfolded but functional Z-A1AT mutant is degraded either by proteasome or by autophagy resulting in reduced secretion, what leads ultimately to lung and liver diseases. The aim of the study is to verify if A1AT/Z-A1AT form a complex with K8 by physical interaction and if this complex regulates A1AT secretion.

Methods: HeLa and CFBE (cystic fibrosis bronchial epithelial) cell lines, transiently transfected with cDNAs of A1ATs (WT or Z mutant) were used 48 h after transfection. The potential interaction between K8 and A1AT was verified by co-immunoprecipitation, and proximity ligation assay (PLA). Secretion of A1AT under different experimental conditions was tested by WB of secreted proteins. The effects of K8 concentration were investigated after silencing K8 expression by transduction of HeLa cells with shRNA. Direct interactions between K8 and A1AT variants were tested by surface plasmon resonance (SPR) on recombinant K8 and purified A1AT from human serum of healthy and ZZ-homozygous patients.

Results: Secretion of Z-A1AT was lower than of WT-A1AT 48h after transfection. WT- and Z-A1AT co-immunoprecipitated with K8 from both HeLa and CFBE cell extracts. PLA experiments showed that Z-A1AT localizes in a close proximity to K8 filaments (< 40 nm) in HeLa and CFBE cells. Number of potential interactions with K8 was higher for Z-A1AT than for WT-A1AT. Biophysical analysis by SPR confirmed that K8 physically interacts with Z-A1AT. K8 filaments network was modified in cells overexpressing Z-A1AT, as compared to WT-A1AT-expressing cells. Reduction of K8 expression in HeLa cells increased the secretion of Z-A1AT.

Conclusions: K8 physically interacts with Z-A1AT. Disruption of this interaction partially restores secretion of Z-A1AT.

We hypothesize that abnormal folding of the Z mutant potentially create cavities which stimulate direct interaction with K8. K8-A1AT complex represents a target for pharmacotherapy of A1AT deficiency. Altogether, K8 is an important regulator of transmembrane (CFTR) and soluble (A1AT) proteins maturation and trafficking in cells.

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preclinical development of a novel corrector for cystic fibrosis

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We have recently identified a corrector (C407), which restores CFTR-Cl⁻ transport in F508del HeLa cells and in primary cultured bronchial CF epithelial cells (Odołczyk *et al.* EMBO Mol Med 2013). We present here data of preclinical development aiming to test if topical and systemic administration of C407 restores CFTR-dependent Cl⁻ transport across nasal and intestinal epithelia.

Methods: 10 μM of C407 was instilled in both nostrils of homozygous F508del FVB mice *cftr*^{tm1Eur} (Van Doorninck *et al.* EMBO journal, 1995). Each mouse was its own control, and was previously instilled with PBS as vehicle.

C407 was injected intraperitoneally (IP) in another group of F508del FVB mice *cftr*^{tm1Eur} mice, 3 times per day for 72 h at a dosage aiming a blood concentration of 10 μM. The control group was treated with the vehicle (PBS).

CFTR function was assessed in nasal epithelial cells after 48 h of treatment using transepithelial potential difference (V_{TE}). Ion transport was evaluated across nasal epithelium by measuring the changes in V_{TE} after perfusion of the nasal mucosa with a low chloride (Cl⁻) solution (Δ Low Cl⁻), followed by the perfusion of Forskolin 10 μM in low Cl⁻ solution (Δ Forskolin) (Saussereau *et al.* Plos One, 2013). Short circuit current (I_{sc}) measurements were performed in distal colon after the last IP injection. The CFTR-dependent Cl⁻ secretion was evaluated as Δ I_{sc} in response to stimulation with 10 μM Forskolin (Fsk) + 100 μM IBMX.

Results:

- C407 administered by nasal instillation restored CFTR-dependent Cl⁻ secretion across the nasal mucosa (mean Δ Low Cl⁻ after treatment (-1.1 ± 2.6) vs mean Δ Low Cl⁻ after vehicle (+1.2 ± 2.0) (p=0.04), n=12 mice)
- C407 administered intraperitoneally did not modify significantly V_{TE} after low Cl⁻ perfusion but significantly hyperpolarized V_{TE} after perfusion of Forskolin in low Cl⁻ solution (mean Δ Forskolin after treatment (-4.3 ± 2.3) vs mean Δ Forskolin after vehicle (-0.8 ± 2.3) (p=0.02), (n=11 treated vs n=5 control).
- CFTR-dependent Cl⁻ current in mice colon was significantly increased after C407 (mean Δ I_{sc} after treatment (13.2 ± 7.0 μA/cm²) vs mean Δ I_{sc} after vehicle (8.7 ± 4.0 μA/cm²), (p=0.04).

Conclusions: The C407 corrector tends to restore CFTR Cl⁻ transport across F508del murine epithelia. Further studies are necessary to determine the optimal dose and pharmacokinetics of this compound.

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Rescue of N1303K chloride currents by small molecule correctors and transcomplementation

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N1303K, associated with severe disease. The goal is to gain more information on the impact of this mutation on CFTR processing and to devise ways to rescue it. Inhibition of proteasomes with MG132 or aggresomes with tubacin is able to rescue the immature band B and mature band C of N1303K, indicating that degradation is via proteasomes and aggresomes. VCP, the protein involved in translocation of mutant proteins to the proteasome and HDAC6, a protein involved in trafficking of mutant proteins to the aggresome, also binds to N1303K indicative of degradation in the proteasome and aggresome. There is no effect of the lysosome inhibitor (E64). We tested the effect of small molecule correctors in promoting maturation of N1303K. We found that several known correctors alone or in combination increased the maturation of B to C band. The best correction occurred when a combination of the CFFT correctors, C4 plus C18 or C3 plus C4 was applied. In pull-down assays, we found that a number of chaperones, including HSP27, 40, 70 and 90 bind to N1303K. Importantly, following treatment with the combination of correctors, chaperone binding was reduced. This strengthens considerably our conclusion, that C3+C4 rescue N1303K by reducing chaperone binding thereby promoting maturation. We next tested whether N1303K could be rescued by transcomplementation. Indeed transfection of the CFTR truncation, $\Delta 27-264$, into cell lines stably transfected with N1303K was able to increase the mature C band at the cell surface. In order to test whether transcomplementation could rescue function, we created a CFBE410- cell line stably expressing N1303K using Flp-In technology, grew them on transwell supports and treated with 10 μ l of AAV1 containing D264 CFTR. 10 mM forskolin was added along with genistein, followed by 10 mM CFTRinh-172 to demonstrate that the currents were from CFTR. We noted a large increase in currents when compared to cells not exposed to virus indicating restoration of chloride currents. This is the first time that the use truncated forms of CFTR proved to be effective in correcting N1303K. Our results suggest that the N1303K mutation has a profound effect on NBD2 processing. Small molecule correctors are able to increase the maturation of band B to C. In addition the truncation mutant, $\Delta 27-264$, is able transcomplement N1303K and rescue mature C band at the cell surface and chloride channel function. Our results suggest that patients bearing either the N1303K may be good candidates for both corrector and gene therapy. Funded by NACF Foundation.

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Mechanism of action of rattlesnake phospholipase A₂ on CFTR dysfunction

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The snake venom secreted phospholipases A₂ (sPLA₂s) from the *Viperidae* family are well-characterized multifunctional proteins which interact with various protein receptors, regulating their activity. We previously reported that a snake venom sPLA₂ (the CB subunit of crotoxin from *Crotalus durissus terrificus*) increases CFTR-Cl⁻ channel current through direct, high affinity binding to the nucleotide binding domain 1 (NBD1) and ΔF508NBD1 of CFTR (Faure et al *Toxicon* 75:212; 2013).

Aim: In an attempt to identify a new class of correctors and modulators of mutated CFTR the aim of the present study was to further investigate the potentiating and correcting activities of CB on CFTR/ΔF508CFTR at the molecular and cellular level and to propose a mechanism of action of CB on CFTR dysfunction.

Methods and results: We tested the correcting effect of CB in ΔF508-HeLa cells using nystatin-perforated whole cell patch clamp measurements. A maturation of fully glycosylated ΔF508 CFTR was detected by immunoblot analysis after treatment of ΔF508-HeLa cells for 24h with 1nM CB. Interactions between NBD1-CB and ΔF508-CB were measured using surface plasmon resonance (SPR) technology and different stabilities of the complexes were determined. The levels of hydrogen-deuterium exchange (HDex SM) of amide protons in peptic peptides from wtNBD1 and ΔF508NBD1 were compared in the absence and presence of CB. Our results show that CB-binding stabilizes a larger fraction of ABCb region and also affects other regions on ΔF508NBD1. Furthermore, recording of the single-CFTR channel inside-out configuration demonstrated the dependence of the opening of the channel on ATP. This dependence was confirmed by HDex SM analysis.

Conclusions: In the present work using patch clamp, SPR competition studies and HDex SM experiments we demonstrated that the CB subunit of crotoxin is a potent potentiator and corrector for ΔF508CFTR. The site of interaction between CB and ΔF508NBD1 mapped by theoretical methods was confirmed by HDex SM experiments. In agreement with SPR competition experiments we propose that CB prevents formation of the pathogenic protein complex keratin 8-ΔF508CFTR and acts as a corrector addressing the mutated channel to the plasma membrane. The CB-ΔF508CFTR complex modifies trafficking, maturation and activity of the misfolded channel.

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Disruption of a PI3K γ /PKA/PDE signaling complex augments cAMP/PKA-signaling and CFTR activity in non-CF and Δ F508-CF airway epithelial cells

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A kinase anchoring proteins (AKAPs) sequester protein kinase A (PKA) in close proximity to its downstream targets, thereby enhancing efficiency and selectivity in cAMP/PKA signaling. We have identified a novel AKAP/PKA signaling complex, wherein the recently identified AKAP phosphoinositide 3-kinase γ (PI3K γ) facilitates the PKA-mediated phosphorylation and activation of cAMP phosphodiesterases 4 (PDE4). Displacement of PKA from the complex using a cell-permeable disruptor peptide (Patent pending N^o: TO2014A001105) attenuates cellular PDE activity and augments cAMP/PKA signaling. This negative feedback loop is critical for the cAMP/PKA-dependent regulation of CFTR in human bronchial epithelial cells. In non-CF cells, treatment with disruptor peptide increased CFTR-dependent short-circuit currents measured in Ussing chambers to more than 50% of the maximal response induced by forskolin. In cells homozygous for Δ F508-CFTR, the most common mutation found in Cystic Fibrosis (CF) patients, addition of the disruptor peptide alone produced minimal channel activation. However, treatment with disruptor peptide strongly amplified CFTR conductance in the presence of CFTR correctors, drugs that increase expression and membrane-localization of CFTR, and CFTR potentiators, drugs that increase channel gating. Biochemical data confirm augmentation of PKA phosphorylation of CFTR as the mechanism of action of the disruptor. Taken together, our results reveal a critical role of the PI3K γ /PKA/PDE4 signaling complex in the regulation of CFTR activity in airway epithelia, and support the development of complex disruptors as potential CF therapeutics.

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Enhanced CFTR modulation with S-nitrosoglutathione reductase inhibitor in combination with CFTR corrector and potentiator

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Recent clinical trials using CF F508del/F508del patients studied the combination of a CFTR corrector (VX-809) and potentiator (VX-770) for improving both CFTR folding/trafficking and channel gating. Although overall effects on lung function (e.g. FEV₁) were statistically significant; they were modest, suggesting the need for additional therapeutic approaches with an alternative mechanism of action. In this pre-clinical study, we assessed the additive effects of a S-nitrosoglutathione reductase inhibitor, N91115 on modulating CFTR function in combination with VX-809 or with VX-809 + VX-770. These in vitro studies were conducted using CF (F508del/F508del) derived primary human airway epithelial (HAE) cells, where short circuit current (I_{sc}) was measured by the Ussing chamber assay. CF HAE cells were seeded onto Snapwell™ inserts and once fully differentiated were treated for 24 h with vehicle (negative control); VX-809 alone (3 μ M), VX-809 + VX-770 (3 μ M for both compounds), and with the addition of N91115 (30 μ M) for the final 4 h of the 24 h incubation period. When N91115 was added to VX-809 (double combination), there was a comparable forskolin-mediated stimulation of F508del CFTR measured in CF HAE cells compared to VX-809 alone. However, after the subsequent addition of VX-770, the double combination produced a greater increase in I_{sc} . This data demonstrated an approximate 30% increase in total potentiated CFTR function by measuring the CFTR-stimulated area under the curve (AUC, a representative indication of CFTR-mediated net chloride secretion). In addition, the change in I_{sc} (ΔI_{sc}) that was inhibited by the addition of the CFTR inhibitor (172) was significantly greater in CF HAE cells exposed to the double combination versus VX-809 alone, further suggesting an additive benefit with N91115. In a second series of studies, CF HAE cells treated with VX-809 + VX-770 showed a significant increase in forskolin-stimulated I_{sc} , however, the forskolin stimulated I_{sc} was not “stable” and, thus, rapidly decreased towards the pre-forskolin level. Although addition of N91115 to VX-809 + VX-770 treatment (triple combination) resulted in a comparable level of initial forskolin CFTR activation, the addition of N91115 appeared to “stabilize” or maintain the level of CFTR activity up to the time of inhibition by 172. A positive change in slope (2.2 fold) was observed reflecting sustained N91115-mediated CFTR function. Slope was calculated from a time point immediately after the maximal forskolin I_{sc} was achieved to a time point immediately before the addition of 172. AUC, measured as the net chloride secretion immediately before the addition of forskolin to 172 inhibition, also demonstrated a significant improvement (1.7 fold increase) with the triple combination versus VX-809 + VX-770 alone. Importantly, these observations were observed within CF HAE cells derived from multiple CF lung codes. Collectively, we demonstrated that N91115 increased and prolonged CFTR activity and functioned as a “stabilizer.” Addition of N91115 to VX-809 and VX-770 may provide a novel combination therapeutic strategy for CF patients.

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Analysis of the chronic effects of lumacaftor and ivacaftor on F508del-CFTR single-channel gating and stability

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Combination therapy with cystic fibrosis transmembrane conductance regulator (CFTR) correctors and potentiators is required to rescue F508del-CFTR, the most common cystic fibrosis (CF) mutation. In recent clinical trials, chronic co-administration of the corrector lumacaftor (VX-809) and the potentiator ivacaftor (VX-770) produced a small, but significant improvement in lung function and improved disease stability¹. However, other studies^{2,3} found that chronic co-administration of lumacaftor and ivacaftor diminishes F508del-CFTR function *in vitro*. In this study, we investigated, at the single-channel level, the effects of chronic co-administration of lumacaftor and ivacaftor on F508del-CFTR using BHK cells expressing F508del-CFTR (F508del-CFTR-BHK cells). We evaluated F508del-CFTR plasma membrane stability at 37 °C by normalizing open probability (P_o) values to those initially recorded at 37 °C after full channel activation (% P_o ; Ref 4).

After chronic treatment with lumacaftor (3 μ M for 24 h at 37 °C), F508del-CFTR channel gating was similar to that of low temperature-rescued F508del-CFTR Cl⁻ channels (P_o ~0.1; n = 7–10) and characterised by a severe gating defect with brief channel openings separated by prolonged channel closures. There was also no difference in plasma membrane stability (% P_o @9 min, ~0%; n = 7–10).

Acute treatment with ivacaftor (10 μ M) restored wild-type levels of P_o to either chronic lumacaftor-treated or low temperature-rescued F508del-CFTR Cl⁻ channels (P_o ~0.45; n = 5–6). The plasma membrane stability of these low temperature-rescued F508del-CFTR channels was worse, with P_o declining to zero quicker than in the absence of ivacaftor. However, there was a small improvement in the plasma membrane stability of the chronic lumacaftor-treated F508del-CFTR channels acutely treated with ivacaftor (% P_o @9 min, 16%; n = 4).

To investigate the impact of chronic co-incubation with lumacaftor and ivacaftor on F508del-CFTR Cl⁻ channels, we incubated F508del-CFTR-BHK cells with lumacaftor (3 μ M) and ivacaftor (1 μ M) for 24 h at 37 °C. Upon activation with PKA and ATP, the single-channel activity of F508del-CFTR was increased greatly (P_o ~0.58; n = 10) and characterised by frequent long channel openings. Of note, the plasma membrane stability of these highly active F508del-CFTR Cl⁻ channels was enhanced markedly

(% P_o @9 min, 67%; n = 8). To try to reconcile these results with previous findings¹⁻³, we studied F508del-CFTR Cl⁻ currents in excised membrane patches from F508del-CFTR-BHK cells chronically co-incubated with lumacaftor (3 μ M) and ivacaftor (1 μ M). Upon activation with PKA and ATP, we observed two populations of F508del-CFTR Cl⁻ current. Most F508del-CFTR current deactivated promptly, but the residual current exhibited delayed deactivation similar to the highly active long-lived F508del-CFTR Cl⁻ channels that we observed in single-channel recordings. We conclude that chronic co-incubation appears to rescue a sub-population of F508del-CFTR Cl⁻ channels. Future studies should identify and characterise these F508del-CFTR Cl⁻ channels.

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References:

- [1] Boyle MP *et al.* Lancet Respir Med. 2014; 2:527-38.
- [2] Cholon DM *et al.* Sci Transl Med. 2014; 6:246ra96.
- [3] Veit G *et al.* Sci Transl Med. 2014; 6:246ra97.
- [4] Wang Y *et al.* Br J Pharmacol. 2014; 171:4490-503.

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Studies of the structural components of VX-770 necessary for its activity

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VX-770 (Ivacaftor) is the first potentiator to be approved for the treatment of patients with cystic fibrosis (CF). To date, it is approved for nine CF-causing mutations including G551D. The drug has also been shown to potentiate wild-type, F508del, and other mutant cystic fibrosis transmembrane conductance regulator protein (CFTR) when added acutely with forskolin, a cAMP activator. However, it was recently reported that chronic treatment with VX-770 in combination with VX-809 (a corrector) has negative effects on cells expressing F508del-CFTR in vitro: the drug abrogates biosynthetic rescue of F508del-CFTR by VX-809. These findings highlight the need to define the binding site for VX-770 on CFTR in order to understand the molecular basis for its positive and negative activities. The objective of our studies is to define the structural basis for VX-770 activity as a potentiator. We are investigating the structure-function relationship of VX-770 by comparing the ability of VX-770 derivatives to potentiate temperature rescued F508del-CFTR in a fluorescence-based assay. First, baby hamster kidney (BHK) cells stably over-expressing F508del-CFTR are subjected to low temperature rescue to enable partial functional rescue to the cell surface. The cells are subsequently loaded with 6-methoxy-N-(3-sulfopropyl)quinolinium (SPQ), a fluorescent and halide sensitive dye. SPQ fluorescence is initially quenched by the presence of basal chloride levels in the cells; after F508del-CFTR channel activation with forskolin, chloride efflux dequenches SPQ fluorescence. The change in fluorescence is measured and used as an indicator of channel activity. Initial studies revealed structural features that are critical for potentiation as well as a region that can be modified without a deleterious effect on activity. The latter finding identifies a potential site where a tag can be incorporated for future studies. Taken together, these preliminary results support the feasibility of our plan to determine the structure-activity relationship of VX-770. The studies may provide us with more information on the structural components of VX-770 necessary for its activity and reveal a position where a photolabel can be incorporated. The resulting chemical probe will be useful for labelling and identifying the VX-770 binding site on CFTR; it may also be used as a tool to elucidate the positive and negative effects of VX-770 on G551D- and F508del-CFTR respectively. Ultimately, the probe may provide us with more information on the potential mechanism of action of VX-770 and enable the development of more effective CF drugs in the future.

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The effect of chronic VX-770 exposure on the plasma membrane stability of VX-809-rescued F508del-CFTR Cl⁻ channels in polarised epithelia

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The most common cystic fibrosis (CF) mutation, F508del, disrupts cystic fibrosis transmembrane conductance regulator (CFTR) function by impairing protein processing, perturbing channel gating and reducing plasma membrane stability. Small molecules called CFTR correctors (e.g. lumacaftor [VX-809]) and CFTR potentiators (e.g. ivacaftor [VX-770]) have been developed to restore function to F508del-CFTR. In recent clinical trials, combination therapy with lumacaftor and ivacaftor had mild clinical benefit improving lung function and disease stability¹. One potential explanation for these clinical data is that chronic co-administration of lumacaftor and ivacaftor decreases the functional expression of F508del-CFTR in human airway epithelia^{2,3}. In this study, we examined the effects of chronic co-administration of lumacaftor and ivacaftor on the plasma membrane stability of F508del-CFTR in polarised epithelia.

Fischer rat thyroid (FRT) cells expressing F508del-CFTR were grown as polarised epithelia and treated with lumacaftor (3 μ M), by itself, or with ivacaftor (1 μ M) for 24 hours at 37 °C. Alternatively, F508del-CFTR expressing FRT epithelia were cultured at low temperature (27 °C) for 48 hours to rescue the plasma membrane expression of F508del-CFTR. At T=0 hours, drug treatments were removed by washing with fresh media and low temperature-rescued epithelia were returned to 37 °C. Ussing Chamber studies were then performed at T=0, 2, 4 and 6 hours in the presence of a basolateral to apical chloride gradient. Transepithelial short circuit current (I_{sc}) was measured in the presence of forskolin (10 μ M) and genistein (50 μ M) to maximally activate CFTR Cl⁻ currents.

At T=0 hours, treatment with lumacaftor corrected the plasma membrane expression of F508del-CFTR to a comparable level as that of low temperature rescue (lumacaftor: I_{sc} , 63 \pm 3 μ A/cm², n=9; low temperature: I_{sc} , 53 \pm 4 μ A/cm², n=11). However, lumacaftor treatment failed to improve the plasma membrane stability of F508del-CFTR with I_{sc} decreasing rapidly by 83% (\pm 1%, n=6) by 2 hours. By contrast, low temperature-rescued F508del-CFTR activity only decreased by 25% (\pm 15%, n=4) at 2 hours. Interestingly, F508del-CFTR FRT epithelia treated with both lumacaftor and ivacaftor for 24 hours had a comparable degree of CFTR rescue to lumacaftor treated epithelia (I_{sc} , 50 \pm 3.2 μ A/cm², n=5), but displayed improved plasma membrane stability when compared to lumacaftor treatment alone, decreasing by only 28% (\pm 6.2%, n=3) over 2 hours. Of note, at T=0 hours, F508del-CFTR FRT epithelia treated with lumacaftor + ivacaftor were maximally activated by forskolin and no further increase in I_{sc} was achieved with genistein addition. We interpret this result to suggest that F508del-CFTR remains fully potentiated by ivacaftor throughout the treatment period.

In conclusion, in FRT epithelia, lumacaftor-corrected F508del-CFTR has reduced plasma membrane stability compared to low temperature-rescued F508del-CFTR. Some stability is restored to lumacaftor-treated epithelia by chronic treatment with ivacaftor. Further studies of the plasma membrane stability of F508del-CFTR are ongoing.

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References:

- [1]: Boyle MP et al. *Lancet Respir Med*. 2014; 2:527-38.
- [2]: Cholon DM et al. *Sci Transl Med*. 2014; 6:246ra96.
- [3]: Veit G et al. *Sci Transl Med*. 2014; 6:246ra97.

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Task force for CF: an Italian drug discovery project to identify novel correctors and potentiators of F508del-CFTR

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Deletion of phenylalanine 508 (F508del), the most frequent mutation among patients with cystic fibrosis (CF), causes a defective maturation of CFTR protein. The maturation defect can be treated with chemical compounds known as correctors. F508del and other mutations like G551D also show a gating defect that requires another type of compounds called potentiators. At the moment there are no F508del correctors with adequate efficacy. Pharmacological rescue of F508del-CFTR function may also benefit from the identification of novel potentiators devoid of the negative effect shown by the new drug VX-770 and other potentiators in recent in vitro studies (Cholon et al., *Sci Transl Med* 6: 246ra96, 2014; Veit et al., *Sci Transl Med* 6: 246ra97, 2014). Indeed, it has been observed that some potentiators have an undesired activity on F508del-CFTR protein processing, resulting in decreased activity of VX-809 as corrector.

To identify drug-like small-molecules that rescue mutant CFTR function, we adopted a high-throughput screening approach. We screened a chemical library (selected according to stringent drug-likeness and maximal diversity criteria) containing 11,334 compounds using two different cell types (FRT and CFBE41o-) expressing F508del-CFTR. For the screening, we used two functional assays based on the halide-sensitive YFP, designed to identify correctors and potentiators. The screening identified:

- i) 104 hits active as potentiators among which 5 compounds with activity comparable to that of VX-770;
- ii) 5 new correctors with activity on both FRT and CFBE41o-cells.

All compounds have been confirmed and characterized with dose-response experiments and cytotoxicity tests.

The effect of selected correctors was analyzed by determining the electrophoretic mobility and the pattern of band B and band C. Some compounds increase both band C and band B, an effect that could be explained with decreased degradation of CFTR protein (or increased CFTR protein synthesis). Other compounds show a prevalent effect on band C which could suggest an improvement in protein stability/maturation. The specificity of correctors was confirmed by using the selective CFTR inhibitor 172 and by testing the correctors on parental cells which have negligible expression of F508del-CFTR.

All potentiators detected in the primary screening were tested at different concentrations on cells expressing F508del- and G551D-CFTR. Interestingly, many compounds are effective on both mutants with a strong increase in CFTR activity that in some cases approaches the effect of VX-770. In particular, one compound has high efficacy and an interesting submicromolar potency. To check whether chronic incubation with potentiators interferes with pharmacological rescue of F508del-CFTR by VX-809, we incubated the cells for 24 hours with compounds together with VX-809. We found that many potentiators indeed decrease the activity of F508del-CFTR. However, other compounds do not display this undesired activity, despite a significant potentiator activity. Such compounds could be considered for the development of potentiators selective for F508del.

The active compounds identified so far will be a starting point for the synthesis of novel potentiators and correctors having improved potency and efficacy.

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The challenge of defining a normal range for transepithelial resistance (TER) in human nasal airway epithelia reconstituted *in vitro* at ALI

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Regulation of osmotic permeability is involved in apical surface liquid homeostasis in lung epithelia

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The apical surface of lung epithelia has to be shielded by the apical surface liquid (ASL). Its volume is adjusted via regulating epithelial ion and water transport. Especially CFTR patients suffer from defective ASL volume homeostasis.

Herein we elucidated mechanisms of ASL volume homeostasis in air/liquid interface cultivated NCI-H441 epithelia and human tracheal epithelia. The novel deuterium oxide (D₂O) dilution method was used to directly quantify water transport and ASL volumes. Ion transport was studied in Ussing chamber experiments. This enabled us to correlate water and ion transport measurements. After perturbation of ASL volume by adding isotonic solution the time course of water transport was measured. In both epithelia, we observed a low resorptive state (lowRS) and a high resorptive state (highRS). The major amount of volume was resorbed during highRS. Switchover from lowRS to highRS was reversible and occurred within 4 to 6 h after ASL volume increase. It did not correlate with the activation of epithelial Na⁺ channels but was accompanied by an increase in osmotic water permeability (P_{osm}). We adapted the standing gradient model to fit epithelial transport on the basis of our measurements. These calculations revealed the increase in P_{osm} as the trigger for highRS onset.

Our data show that ASL volume is adjusted not solely by regulating ion transport. The regulation of P_{osm} is an additional and limiting mechanism in lung epithelia to respond to an impaired ASL volume.

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Cellular volume regulation by CFTR, LRRC8A, and TMEM16 proteins: a network

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Volume regulation is an intrinsic property of any living cell. An increase in intracellular osmolytes or decrease in extracellular osmolarity leads to cell swelling, which is counteracted immediately by activation of K^+ and Cl^- currents, releasing KCl to the extracellular space. CFTR has been well recognized as a Cl^- channel controlling cellular volume, which in turn affects transport by other membrane proteins. In epithelial cells opening of apical CFTR Cl^- channels leads to a sudden drop in cell volume, as Cl^- is above its electrochemical equilibrium and therefore leaves the cell. Although CFTR's transport activity affects cell volume, it remains controversial, whether CFTR is actually activated during cell swelling and therefore contributes to cellular volume regulation. Recently LRRC8A has been identified as a ubiquitous and essential component of the volume regulated chloride channel (VRAC), a biophysical entity that is believed to be essential for volume regulation. VRAC has been shown earlier to be inhibited by CFTR. When we expressed CFTR in HCT116 human colon carcinoma cells completely lacking expression of LRRC8A (LRRC8A^{-/-}), we detected pronounced swelling activated whole cell currents, which were significantly larger than in LRRC8A^{+/+} cells. Notably, we found that LRRC8A and CFTR can be coimmunoprecipitated when coexpressed in HEK293 cells.

TMEM16A (anoctamin 1) is the Ca^{2+} activated Cl^- channel widely expressed in epithelial organs and HCT116 cells, together with other TMEM16 proteins. TMEM16A currents activated through stimulation with ATP (Ca^{2+} increase) were again significantly smaller in LRRC8A^{+/+} when compared to LRRC8A^{-/-} cells. Similar was observed when TMEM16A was expressed in HEK293 cells: Coexpression of LRRC8A attenuated ATP-activation of TMEM16A, while ATP-activated TMEM16A currents were reduced after siRNA-knockdown of LRRC8A in CAL-27 cells. Also TMEM16A and another member of the TMEM16 family, TMEM16K could be coimmunoprecipitated together with LRRC8A. For TMEM16A a role for cellular volume regulation has been demonstrated earlier. Using various mammalian cell models and *Xenopus* oocytes, we found that expression of TMEM16K was clearly correlated with swelling activated whole cell currents and regulatory volume decrease. CFTR and TMEM16A have been shown to interact functionally: cAMP-dependent activation of CFTR currents abrogates Ca^{2+} -dependent activation of TMEM16A. As we also coimmunoprecipitated TMEM16A with CFTR, we suggest a functional and protein-network of CFTR, TMEM16 proteins and LRRC8A. While volume regulation can occur in the absence of LRRC8A, the definite role of these putative channels components as well as CFTR and TMEM16 for cellular volume regulation in native tissues remains to be demonstrated.

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Assessing global cellular responses to CFTR misfolding in laboratory cell lines and patients using RNA sequencing and ribosome profiling

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Misfolding, misprocessing and degradation of CFTR caused by a deletion of F508 contributes to 90% of CF incidences. Conflicting views exist on the effect of CFTR misfolding on cellular homeostasis and evidence ranges from CFTR misfolding activating the cellular stress response to being rather completely uncoupled from it. We therefore used ribosome profiling and RNA-sequencing to gain insights into the global impact of $\Delta F508$ CFTR misfolding on translational and transcriptomic landscape and specifically to explore its effect on the ER-folding machinery and its ability to activate the unfolded protein response (UPR) by applying ribosome profiling and RNA-sequencing. While RNA-sequencing yields information on RNA abundance and thus transcriptional regulation, ribosome profiling reveals the position of translating ribosomes with nucleotide resolution and therefore allows assessment of protein biosynthesis. We analyzed CFBE cells expressing WT and $\Delta F508$ CFTR and samples derived from lung tissue of CF patients. In general, we found a high correlation between gene expression levels of WT and $\Delta F508$ CFTR expressing cells indicating minor changes in response to CFTR misfolding. Target genes of the UPR were not activated, rather genes participating in cell contact or cytoskeletal architecture were transcriptionally upregulated. Furthermore, the key components of the ER stress response, e.g. XBP1-splicing and translational activation of ATF4, were not activated in response to $\Delta F508$ CFTR expression in CFBE cells implying lack of induction of the UPR. Intriguingly, in the patients we observed XBP1 splicing and induction of down-stream genes clearly indicating an ER-mediated stress response. Reshaping the ER-folding machinery may increase the cellular capability to counteract ER-stress imposed by $\Delta F508$ CFTR or may contribute to the maladaptive stress response.

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Developing a standardized protocol for ratiometric measurement of CFTR-dependent secretion by human sweat glands

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A bioassay for testing the CFTR function *in vivo* was recently published by Wine et al, (Plos ONE 2013 and 2014). A ratio was computed between CFTR-independent (M-sweat, stimulated by methacholine) and CFTR-dependent (C-sweat, evoked by a β -adrenergic cocktail) sweat secretion rates by multiple individual glands, measured in a series of subjects. Secretion rates were given by changes of volume of sweat drops secreted in an oil layer, including the presence of a water-soluble dye for the C-sweat. Multicenter validation for diagnosis and detection of CFTR function improvement during treatments with drugs targeting the basic defect require a Standardized Operating Procedure (SOP).

We have been focusing on developing a simplified method suitable for implementation in clinical multicentric trials, in collaboration with J.W. (Stanford, USA), and on setting up the method in Brussels (T.L.).

Specific concerns were commercial availability of instruments and reducing their costs. We are now collecting reference data in non-CF subjects, patients with classical or non-classical forms of CF and carriers. We aim at automating the analysis procedure originally performed using the ImageJ software. We reproduced the test at the CF Center of Verona, simplified the procedure and acquired images for developing the image analysis software in collaboration with researchers (M.T., S.C.) of the Politecnico di Milano (Italy).

Standardization of a protocol for testing CFTR function *in vivo* that is complementary to sweat test procedures already available will contribute to multicenter clinical studies as well as to define controversial diagnosis.

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Constitutive activation of the Ca²⁺-activated chloride channel TMEM16A

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TMEM16A protein (also known as anoctamin-1) is a calcium-activated chloride channel expressed in epithelial cells of different organs. In the airways, TMEM16A is localized on the surface epithelium and the sub-mucosal glands, where it is abundantly expressed in mucous cell, particularly under inflammatory conditions. In airway epithelia, the transient transepithelial chloride transport elicited by purinergic agonists is significantly reduced after knockdown of TMEM16A expression. Therefore, TMEM16A may represent an alternative pathway to circumvent the reduced epithelial anion transport in cystic fibrosis. Importantly, this approach could be applied to all patients irrespective of their genotype. Thus, the molecular mechanisms of underlying TMEM16A activity should be investigated, to identify approaches that may lead to a persistent channel activation, possibly without requiring the increase in intracellular calcium. Indeed, we have previously shown that the replacement of the TMEM16A carboxy-terminus with the same region of TMEM16B generates constitutively active channels. Therefore, we hypothesized that the insertion of TMEM16B-specific amino acid residues into the TMEM16A C-terminus perturbs its interaction with another intracellular domain of the protein, causing a significant conformation change that promotes persistent channel pore opening. Accordingly, we decided to investigate this phenomenon to understand if a similar persistent opening could be obtained with a drug that modifies TMEM16A interdomain interactions.

In a series of site-directed mutagenesis and functional studies (HS-YFP assay and patch-clamp electrophysiology), we restricted the activating domain of TMEM16B to a sequence of 10 residues in which only 4 residues are different with respect to the native TMEM16A C-terminus. We also studied the interaction of the “activating” C-terminus with other regions of TMEM16A. In particular, we replaced other domains of the “activated” TMEM16A protein with the corresponding regions of TMEM16B to generate double chimeras. We found that one of these double chimeric constructs is devoid of constitutive channel activity thus revealing the domain that interacts with the C-terminus.

In conclusion, we have elucidated the type of domain-domain interactions that in chimeric TMEM16A lead to constitutive activation. Finding the minimal modifications of TMEM16A sequence that lead to constitutive activity and clarifying the structural implications of such modifications could pave the way for the rational design of novel TMEM16A activators.

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TMEM16A is required for intestinal Cl⁻ secretion and activation of CFTR

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CFTR and Ca²⁺ activated chloride channels were seen traditionally as independent chloride channels located in the apical membrane of epithelial cells. However, earlier studies showed larger, yet more transient Ca²⁺, i.e. UTP- activated Cl⁻ secretion in human nasal tissues of patients lacking expressing of functional CFTR. Later studies demonstrated that Ca²⁺ dependent Cl⁻ currents by TMEM16A could no longer be activated after CFTR was fully stimulated by IBMX/forskolin. It was shown that this effect is reversible upon washout of IBMX/forskolin, however, the mechanism remained obscure. From earlier quenching experiments using yellow fluorescent protein, we detected a significant increase in the intracellular Cl⁻ concentration by about 10 mM, when CFTR-expressing cells were stimulated with IBMX/forskolin. Notably, recent patch clamp experiments with TMEM16-expressing HEK293 cells suggest an inhibition of TMEM16 currents by elevated levels of intracellular Cl⁻.

More recently a considerable overlap was noticed between both intracellular cAMP and Ca²⁺ signaling pathways: Intracellular Ca²⁺ not only stimulate basolateral K⁺ channels and thereby supplies the driving force for apical Cl⁻ secretion, but also activates CFTR through inhibition of phosphatases and increase of protein kinase C activity. In order to further unravel the relationship between cAMP- and Ca²⁺- dependent Cl⁻ secretion, we selectively knocked down TMEM16A and TMEM16K in TMEM16A-floxed and TMEM16K-floxed mouse intestine, using Cre recombinase under the control of a villin promotor. We found that knockdown of TMEM16A largely reduced CFTR-dependent Cl⁻ secretion stimulated by IBMX/forskolin in ileum and proximal colon, as measured in perfused Ussing chambers. In mouse jejunum, where no TMEM16A expression was detected, knockdown of TMEM16K inhibited Cl⁻ secretion by CFTR.

Furthermore, we freshly isolated epithelial cells from mouse colonic crypts and found largely reduced IBMX/Forskolin-activated CFTR Cl⁻ currents in colonic crypt cells from TMEM16A-knockout animals. Moreover, interference of TMEM16A- and CFTR-dependent Cl⁻ currents was also detected in Baby Hamster Kidney (BHK) cells stably expressing wt-CFTR, but not in cells expressing F508del-CFTR. While the relationship between CFTR and TMEM16A and CFTR and TMEM16K is currently incompletely understood, it is obvious that these Cl⁻ transporting proteins do not operate independently.

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SLC26A9 chlorid channels: generation and functional characterization of stably-overexpressing FRT epithelial cells

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Mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene impair Cl⁻/fluid secretion across airway epithelia causing airway surface dehydration, airway mucus obstruction and chronic lung disease in patients with cystic fibrosis (CF). Previous results in mice revealed that SLC26A9, a recently identified epithelial chloride channel, is essential for preventing airway obstruction associated with IL13-induced airway inflammation (Anagnostopoulou P. et al. *J Clin Invest.* 2012). Further, it was shown that SLC26A9 contributes to cAMP-dependent Cl⁻ secretion and functionally interacts with CFTR in human bronchial epithelial cells (Bertrand et al. *J Gen Physiol.* 2009). These results suggest that SLC26A9 may be a promising alternative Cl⁻ channel in CF. We therefore generated and characterized novel epithelial cell lines with stable expression of SLC26A9 as an epithelial model for studies of regulation and pharmacological activation.

Fisher rat thyroid (FRT) cells were used as an *in vitro* epithelial cell model and stably transduced with a HA-tagged SLC26A9 construct. Expression of SLC26A9 was analysed on mRNA and protein level. Transepithelial Cl⁻ currents were measured by applying a Cl⁻ gradient in Ussing chambers. Moreover, the effects of Cl⁻ channel blockers were evaluated. Further, whole-cell patch clamp experiments were performed. The effect of knockdown of osmotic-stress sensing kinases (e.g. WNK1/4, SPAK) on Cl⁻ currents was studied.

SLC26A9 expression in transduced FRT (FRT-SLC) cells was found at high levels. Immunofluorescence revealed that SLC26A9 was mainly stored in the Golgi-complex. In biotinylation assays, a fraction of apically localized SLC26A9-protein was found. Treatment with Endoglycosidase-H did not result in a mobility shift of the protein. Transepithelial measurements revealed that the basal short circuit current (I_{sc}) was significantly increased in FRT-SLC ($12.3 \pm 2.0 \mu A/cm^2$, $P < 0.01$) compared to control-transfected FRT (FRT-CTL; $3.9 \pm 0.5 \mu A/cm^2$) cell monolayers. cAMP (IBMX/forskolin)-stimulated Cl⁻ secretion was significantly increased in FRT-SLC compared to FRT-CTL cells ($\Delta I_{sc} = 4.9 \pm 0.5$ vs. $\Delta I_{sc} = 0.7 \pm 0.2 \mu A/cm^2$, $P < 0.01$). SLC26A9-mediated currents were inhibited by 50 μM GlyH-101 or 100 μM niflumic acid, but not by 20 μM CFTRinh-172. Whole-cell patch clamp recordings showed a constitutive Cl⁻ current in FRT-SLC cells that was not affected by the application of forskolin. The whole-cell Cl⁻ conductance, however, was significantly blocked by GlyH-101 (48.8 \pm 3.5%) and niflumic acid (44.7 \pm 2.6%). In RNAi studies, knock-down of WNK1 in FRT-SLC epithelial monolayers diminished the basal I_{sc} to 81.6 \pm 7.4% compared to control-treated FRT-SLC cells. Moreover, cAMP-mediated Cl⁻ currents were also significantly reduced after knockdown of WNK1 ($\Delta_{cAMP} = 5.9 \pm 0.7 \mu A/cm^2$, $P < 0.01$) and SPAK ($\Delta_{cAMP} = 8.8 \pm 0.9 \mu A/cm^2$, $P < 0.01$) in comparison to control-treated FRT-SLC epithelial monolayers ($\Delta_{cAMP} = 12.1 \pm 1.4 \mu A/cm^2$).

We established novel stable HA-tagged SLC26A9-overexpressing FRT epithelial cells for studies of SLC26A9 Cl⁻ channel function and regulation. In FRT-SLC cells, SLC26A9 contributes to constitutive and cAMP-stimulated Cl⁻ currents that are regulated by osmotic stress-sensing kinases, in particular WNK1 and SPAK. Cl⁻ channel inhibitors GlyH-101 and niflumic acid, but not CFTR172 inhibit SLC26A9-mediated Cl⁻ currents. This cellular model may be useful for further studies exploring SLC26A9 as an alternative Cl⁻ channel in CF and potentially other muco-obstructive diseases.

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Decreased CFTR activity after ethanol consumption and in alcoholic pancreatitis

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Introduction: Excessive ethanol consumption is one of the most common causes of acute and chronic pancreatitis. It is also documented that genetic defects of CFTR can lead to pancreatitis, however, the role of CFTR in the pathogenesis of alcohol-induced pancreatitis has not been investigated.

Methods: We examined the effects of ethanol, fatty acids and fatty acid ethyl esters on CFTR function and expression in human (volunteers, patients and cell lines) and in animal models (guinea pigs and CFTR knockout mice).

Results: Sweat chloride concentration was increased in patients suffering in alcohol abuse but not in healthy volunteers, indicating alcohol-induced impaired CFTR function. Marked loss of CFTR expression was found in pancreas specimens from patients with acute or chronic alcohol-induced pancreatitis. In functional studies, we detected strong inhibitory effects of alcohol and fatty acids on fluid and HCO₃⁻ secretion and CFTR activity in pancreatic ductal epithelial cells *in vivo* and *in vitro* mediated by sustained intracellular calcium overload, cellular cAMP and ATP depletion and mitochondrial membrane depolarization. We reproduced the alcohol-induced decrease in CFTR expression *in vivo* and *in vitro*, which was caused by a combination of reduced CFTR mRNA levels, decreased cell surface stability and endoplasmic reticulum folding defect of CFTR. Finally, genetic deletion of CFTR lead to more severe pancreatitis in mice induced by ethanol and fatty acid.

Conclusions: The findings indicate that alcohol-induced loss of CFTR function is critical in the development of alcoholic pancreatitis; therefore, correcting CFTR function should offer significant therapeutic benefit.

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Simple method to measure CFTR-dependent iodine transport in peripheral blood leukocytes

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Background: Leukocytes are increasingly recognized in the scientific literature as key component of the pathogenetic events associated to cystic fibrosis. Recent studies performed by our group demonstrated that the Cystic Fibrosis Transporter protein (CFTR) is expressed and functional in peripheral blood monocytes.

Hypotheses and objectives: We hypothesize that peripheral blood leukocytes represent a suitable and easily accessible source of primary cells expressing CFTR that can be exploited to monitor CFTR expression and activity. Our goal is to devise new procedures and methods to monitor CFTR expression and function in leukocytes.

Methods: Leukocytes from control and CF patients were used. Halide sensitive yellow fluorescence protein (HS-YFP) was purified from HS-YFP-transfected FRT cells with a 30% yield and a purification factor of 21.1. Purified HS-YFP shows a K_d for iodide approximately 20-fold higher than for chloride.

Assay of CFTR activity. Cells were washed twice with 0.25 M sucrose, containing 20 mM sodium borate, pH 7.5, and 0.2 mM CaCl₂, suspended at 7.5×10⁶/mL in CFTR buffer, and exposed for 30 min at 37°C to vehicle or 100 μM dibutyl-cAMP and 50 μM of the CFTR potentiator genistein. Cells were then incubated for 20 seconds with 5 mM NaI and supernatant collected after a brief centrifugation step. 5 μl of purified HS-YFP were added to 95 μL of the clear supernatants. Fluorescence was measured at λ_{ex} = 485±15 nm and λ_{em} = 535±10 nm. The fluorescence recovery of HS-YFP is directly related to the internalized I⁻.

Results: Our preliminary data on 20 CF cases in comparison with 24 controls confirm that YFP assay can detect differences in iodine exchange among healthy and CF individuals. The values expressed as mean±SEM are the following: Healthy (283.7±35.1); CF 2nd class (26.7 ± 9.8); other mutations (35.1 ±19.3); maximum signal: YFP (768.4 ± 59.5).

Conclusions: These results lay the basis for the measure of the effect of CFTR correctors/potentiators on CFTR function measured in blood cells of patients in a personalized manner with a relatively simple multiwell platform assay that can be analyzed in a commonly available automated plate reader.

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CFTR is required for mediation of pro/anti-inflammatory balance by vardenafil in mouse CF macrophages

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Lung inflammation is a key feature of cystic fibrosis. Promising anti-inflammatory properties have been reported for vardenafil, a phosphodiesterase type 5 inhibitor (iPDE5). We have recently demonstrated that it reduces LPS-induced inflammatory responses in CF mice (Lubamba et al., 2012). We hypothesized that CF macrophages are characterized by a proinflammatory phenotype which can be modulated by vardenafil. Moreover, we want to examine a possible role of CFTR in vardenafil-induced immunomodulation of macrophages.

Macrophages were isolated and purified from lung homogenates from homozygous F508del-CFTR, CFTR^{-/-} (KO) and wild-type (WT) mice. To test the hypothesis that the activity of macrophages is altered in F508del-CF and KO mice, macrophages differentiation in pro-inflammatory (M1) effectors was studied after polarization with LPS and IFN- γ . Pro-inflammatory mediators TNF- α and NOS-2 were quantified by ELISA (in culture supernatants) or by quantitative RT-PCR (in cultured macrophages). In each condition, the effect of vardenafil (10-50 μ M) was evaluated.

F508del-CF lung macrophages displayed an exaggerated pro-inflammatory response to M1 mediators. Both TNF- α and NOS-2 levels were more than doubled. Similar observations were made in macrophages isolated from KO mice, confirming that loss of CFTR promotes proinflammatory phenotype in macrophages. In F508del-CF mice, vardenafil reduced the expression of pro-inflammatory mediators by at least 50%. However, vardenafil failed to normalize TNF- α and NOS-2 expression in KO macrophages, suggesting that the presence of CFTR protein is required for immunomodulation by vardenafil.

Taken together, our results indicate that macrophages display a pro-inflammatory profile and play a critical role in inflammatory responses in CF. Moreover, the immunomodulatory effect of vardenafil, which could thus be beneficial in CF pharmacotherapy, requires CFTR expression.

Lubamba, B., Huaux, F., Lebacq, J., Marbaix, E., Dhooghe, B., Panin, N., Wallemacq, P., and Leal, T. (2012). Immunomodulatory activity of vardenafil on induced lung inflammation in cystic fibrosis mice. *Journal of Cystic Fibrosis* 11, 266-273.

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Generation of physiologically relevant model of cystic fibrosis airway cells using gene editing of human embryonic stem cells

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Fully differentiated primary human airway epithelial cultures have enabled major advances in our understanding of cystic fibrosis (CF) airway pathogenesis and in subsequent drug discovery efforts. However, CF research is impeded by the limited availability and high variability of patient cells, so that a sustainable model for CF research would be of great benefit to the CF research community as a whole. To this end we propose using designer nucleases to introduce the most common CF mutation, F508del, into an established human embryonic stem cell (hESC) line (CA1), which can then be differentiated into CF relevant cell types such as airway epithelial cells (1).

Transcription activator-like effector nucleases (TALENs) are designer nucleases that can be engineered to bind to any DNA sequence and introduce a double-strand break (DSB) at the target site. In the presence of an homologous DNA template spanning the target site sequence, the DSB increases the efficiency of homologous recombination (HR), which can be used to introduce a desired sequence into the genome. Our approach relies on using TALENs for the introduction of the F508del mutation into the hESC line CA1. The TALEN-treated stem cells will be clonally expanded and screened for the presence of the mutation. Correctly modified CA1 cell clones will then undergo directed differentiation into airway epithelial cells by carefully timed treatment with exogenous growth factors that mimic endoderm developmental pathways *in vivo*, followed by air-liquid interface culture, using a recently established differentiation protocol (1). Differentiated cells will then be analysed biochemically and functionally for the production of the mutant CFTR protein.

Using Golden Gate cloning we have engineered CFTR-specific TALENs, which will be used for the generation of the CF model. To validate the efficiency of these TALENs, we have used a human keratinocyte line, which has previously been used in our laboratory to test a large variety of TALENs. Human keratinocytes were transiently transfected with plasmids encoding TALENs. 72 hours after transfection a T7 endonuclease I assay was performed, which showed that the CFTR-specific TALENs are functional. We designed several DNA templates of different size harbouring the F508del mutation, which will be co-transfected together with the CFTR-specific TALENs to initiate HR at the target site. Once successful target site modification is achieved in our established test system the method will be applied to CA1 hESCs.

By using this method we aim to generate a fully functional airway epithelium that can be grown indefinitely and used as 'model' of CF cells. Furthermore, the original unmodified hESC line provides an unlimited source for a perfect 'healthy control'. This model will be suitable for physiological CF research, drug screening and drug therapy evaluation, and it obviates many of the problems associated with primary cells.

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1. Wong AP, Bear CE, Chin S, Pasceri P, Thompson TO, Huan LJ, Ratjen F, Ellis J, Rossant J. Directed differentiation of human pluripotent stem cells into mature airway epithelia expressing functional CFTR protein. *Nat.Biotechnol.* 2012;30:876-82