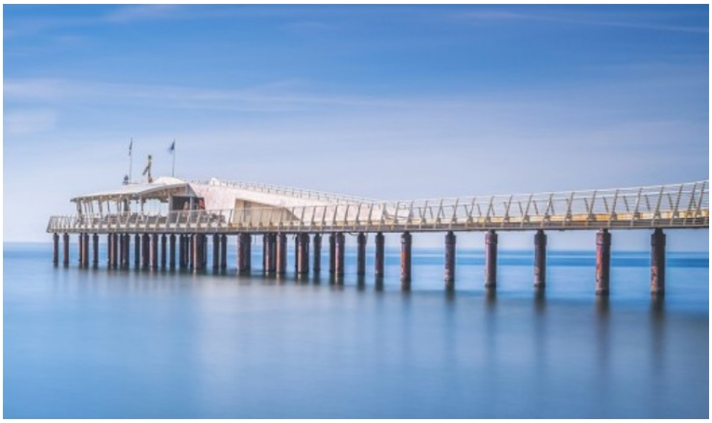
2025

European Cystic Fibrosis Society

20th ECFS Basic Science Conference

Conference Programme & Abstract Book

Lido di Camaiore



Chairpersons

Patrick Harrison, Batsheva Kerem and Dean Madden

26 March – 29 March 2025

CONTENTS

	Page
Conference Sponsors	5
Conference Programme	11
Poster Titles & Authors	15
Award Winners	21
Session Abstracts	23
Poster Abstracts	72

CONFERENCE SPONSORSHIP & SUPPORT

The ECFS thanks the following for their support

WELCOME FROM THE ECFS PRESIDENT

This year we are delighted to welcome Dr. Patrick Harrison (US) as the conference Chairperson who will be supported by Prof. Batsheva Kerem (IL) and Dr. Dean Madden (US) as co-chairpersons.

Basic scientific discovery is critical to our understanding of CF. New therapies targeting the basic defect have been an extraordinary achievement coming from basic scientific research. But there are still many critical questions left unanswered and basic science in CF is needed more than ever to cure all patients with CF.

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

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

A very warm welcome to an exciting conference.

Jane Davies
President
European Cystic Fibrosis Society

WELCOME FROM THE CONFERENCE CHAIRPERSONS

We are very happy to welcome you to the 20th Basic Science of CF conference 2025, taking place this year in Lido di Camaiore, Tuscany, Italy. Located on the Versilian coast, Lido di Camaiore offers a beautiful seaside setting nestled between the Apuan Alps and the Ligurian Sea. This charming town provides a relaxing atmosphere and serves as an excellent hub for exploring the nearby Italian cities, the beautiful hills, and the enchanting landscapes of the wider Tuscan region.

With this conference, you can expect to hear about recent unpublished results and engage in discussions of data and ideas in an informal and interactive environment.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 the field.

This year's programme features eight symposia with invited speakers and talks from submitted abstracts. There are also two keynote lectures and flash poster sessions. This conference will be a unique chance to discuss topical aspects of basic research in cystic fibrosis with international experts.

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.

Patrick Harrison
Cincinnati Children's Hospital,
Cincinnati, OH
USA

Batsheva Kerem
The Hebrew University, Jerusalem
Israel

Dean Madden
Geisel School of Medicine at
Dartmouth, Hanover, NH
USA

20th ECFS Basic Science Conference

26 – 29 March 2025, Lido di Camaiore, Italy

Programme

Chairpersons:
Dr. Patrick Harrison - Cincinnati Children's Hospital, Cincinnati, OH, USA.
Prof. Batsheva Kerem - The Hebrew University, Jerusalem, Israel
Dr Dean Madden - Geisel School of Medicine at Dartmouth, Hanover, NH, USA

Wednesday, 26 March 2025 (Day 1)	
17:30-18:00	**Official Opening of the Conference by the Conference Chairpersons**
	20 years of ECFS Basic Science Conference – Margarida Amaral (PT)
18:00-19:00	**Opening Keynote Lecture:** CFTR, from folding to function - Ineke Braakman (NL)
19:00-19:45	**Welcome Reception**
19:45-21:30	*Dinner*

Thursday, 27 March 2025 (Day 2)	
07:30-08:45	*Breakfast*
08:45-10:30	**Symposium 1 – Therapeutic approaches for nonsense and splicing mutations** **Chairs: Batsheva Kerem (IL) / John Lueck (US)**
08:45-09:10	Fighting the nonsense. From basic science to new drugs - Isabelle Sermet-Gaudelus (FR)
09:10-09:35	Premature stop-codon readthrough by R-ASO - Andrei A. Korostelev (US)
09:35-10:00	Development of a platform therapeutic for nonsense-associated cystic fibrosis - John Lueck (US)
10:00-10:10	Abs. 10 - Identification of novel pharmacological modulators of the NMD mechanism to recover CFTR function in patients with nonsense mutations - Arianna Venturini (IT)
10:10-10:20	Abs. 08 - Developing novel ribosome-directed small molecules and antisense oligonucleotides as therapeutic approaches for refractory CFTR variants - JaNise Jackson (US)
10:20-10:30	Abs. 06 - Nonsense suppression of CFTR PTC variants through attenuation of translation termination and mTOR inhibition - Feng Liang (US)
10:30-11:00	*Coffee break* & **Poster viewing**
11:00-12:45	**Symposium 2 – Mucus and mucins in CF**
	Chairs: Camille Ehre (US) / Noel G McElvaney (IE)
11:00-11:25	Mucins under pressure: the key to understanding mucus stasis in CF- Camille Ehre (US)
11:25-11:50	Changing inflammatory profiles post Trikafta - Noel G McElvaney (IE)
11:50-12:15	Impact of inflammation on airway mucus properties and clearance - Marcus Mall (DE)
12:15-12:25	Abs. 19 - *Ex vivo* pig lung as a new cystic fibrosis model for the study of *Pseudomonas aeruginosa* biofilm infection and phage therapy application - Marco Cafora (IT)

12:25-12:35	Abs. 16 - Investigating the therapeutic effects of K+ channel modulators on mucus and airway surface liquid in cystic fibrosis - Omar Hamed (UK)
12:35-12:45	Abs. 20 - Utilisation of human induced pluripotent stem cells for modelling cystic fibrosis lung disease in vitro - Mark-Christian Jaboreck (DE)
12:45-14:30	*Lunch*
14:30-15:30	**Flash Poster Session (even numbers)** **Chair: Nicoletta Pedemonte (IT)**
15:30-15:45	The Italian CF Research Foundation: funding strategies and outcomes - Ermanno Rizzi (IT)
15:45-16:15	*Coffee break* & **Poster viewing**
16:15-18:00	**Symposium 3 – CFTR and cancer / ageing** **Chairs: Patrick Harrison (US) / Sacha Spelier (NL)**
16:15-16:40	Cystic fibrosis associated colorectal cancer; a complex and urgent challenge to tackle - Sacha Spelier (NL)
16:40-17:05	Growing older with CF: new challenges for CF care and research – Isabelle Fajac (FR)
17:05-17:30	Cardiovascular disease and CF; Should we be concerned? – Damian Downey (UK)
17:30-17:40	Abs. 25 - Elexacaftor/tezacaftor/ivacaftor CFTR modulators mitigate senescence in cystic fibrosis - Anca Manuela Hristodor (IT)
17:40-17:50	Abs. 22 - Pancreatic cancer growth rate may be susceptible to variations in CFTR expression - Raquel Ibañez (ES)
17:50-18:00	Abs. 21 - Development of new models to study the impact of CFTR dysfunction on osteocytes in cystic fibrosis-related bone disease - Léa Thoraval (FR)
20:00-21:30	*Dinner*
21:30-23:00	**Evening Poster Session:** Posters with even numbers
Friday, 28 March 2025 (Day 3)	
07:30-08:45	*Breakfast*
08:45-10:30	**Symposium 4 – CFTR structure and function and therapeutic relevance** **Chairs: Dean Madden (US) / Margarida Amaral (PT)**
08:45-09:10	Elexacaftor-tezacaftor-ivacaftor rescue of F508del-CFTR chloride channels - David Sheppard (UK)
09:10-09:35	Understanding and fostering CFTR potentiation - Isabelle Callebaut (FR)
09:35-10:00	Structure/function mechanisms of CFTR modulators - TC Hwang (US)
10:00-10:10	Abs. 54 - A novel active conformation of CFTR may drive functional recovery of F508del-mutant - Cédric Govaerts (BE)
10:10-10:20	Abs. 30 - *In silico*, *in vitro* and *ex vivo* characterization of CFTR pathogenic variants localized in the Fourth Intracellular Loop and their rescue by modulators - Emanuela Pesce (IT)
10:20-10:30	Abs. 34 - Novel class 3 CFTR correctors suitable for combinatorial treatments in cystic fibrosis - Marilia Barreca (IT)
10:30-11:00	*Coffee break* & **Poster viewing**

11:00-12:45	**Symposium 5 – CFRD from different perspectives: How do pwCF, clinicians, researchers deal with diabetes in CF?** A patient organisation-initiated symposium
	Chairs: Paula Sommer (UK) / Isabelle Sermet-Gaudelus (FR)
11:00-11:15	*Patient view video:* Perspectives of people with CF across Europe on the impact of CF diabetes on their lives
11:15-11:45	CFRD genetics: from pathophysiology to personalized medicine - Scott Blackman (US)
11:45-12:05	CFRD in adult care / case report demonstrating demands to improve care - Bibi Uhre Nielsen (DK)
12:05-12:25	Changing nutritional trends in people with CF – what is the impact on management of CF related diabetes? – Joanna Snowball (UK)
12:25-12:45	Organ on chip modelling of beta cell function/ pancreatic exocrine-endocrine interface/potential utility of cell therapies - Victoria Salem (UK)
12:45-14:00	*Lunch*
14:00-18:30	**Free Afternoon**
18:30-19:30	**Flash Poster Session (odd numbers)** **Chair: Margarida Amaral (PT)**
20:00 -21:30	*Dinner*
21:30-23:00	**Evening Poster Session:** Posters with odd numbers
Saturday, 29 March 2025 (Day 4)	
07:30-08:45	*Breakfast*
08:45-10:30	**Symposium 6 – Lung regeneration**
	Chairs: Amy L Ryan (US) / Marta Vilà González (ES)
08:45-09:10	iPSC-derived lung and pancreatic cells: unlocking regenerative therapies for cystic fibrosis - Amy L Ryan (US)
09:10-09:35	Transplantation of autologous epithelial cells to airways - Robert E. Hynds (UK)
09:35-10:00	hiPSC-derived airway epithelium: exploring CF physiopathology and regenerative strategies - Marta Vilà González (ES)
10:00-10:10	Abs. 61 - Mitochondrial metabolic rewiring drives wound healing in cystic fibrosis primary airway epithelium cultures treated with Elexacaftor/Tezacaftor/Ivacaftor - Mairead Aubert (FR)
10:10-10:20	Abs. 58 - Unveiling new insights of ionocytes' role in cystic fibrosis using single-cell RNA sequencing analysis and human induced pluripotent stem cell-derived airway epithelial cells - Míriam Salvà Barceló (ES)
10:20-10:30	Abs. 59 - GY971, a new anti-inflammatory agent for the treatment of Cystic Fibrosis lung disease - Ilaria Lampronti (IT)
10:30-11:00	*Coffee break* & **Poster viewing**

11:00-12:45	**Symposium 7 – Gut microbiome, gut-lung axis**
	Chairs: Damian Downey (UK) / Laurence Delhaes (FR)
11:00-11:25	A role for the gut-lung axis in regulating chronic infections in CF - Jennifer Bomberger (US)
11:25-11:50	Impacts of CF therapies on the gut microbiome - Chris van der Gast (UK)
11:50-12:15	Lessons from the gut-lung axis in the CFTR modulator era - Laurence Delhaes (FR)
12:15-12:25	Abs. 65 - The gut's role in pulmonary and extrapulmonary manifestation of Cystic Fibrosis - Alessandra Bragonzi (IT)
12:25-12:35	Abs. 64 - Gut microbiota-metabolome relationships across pwCF taking highly effective CFTR modulators: Preliminary findings from the GRAMPUS-CF study - Ryan Marsh (UK)
12:35-12:45	Abs. 63 - Modeling a Triple Culture Airway-on-a-Chip for Inflammation Studies in Cystic Fibrosis - Roberto Plebani (IT)
12:45-14:15	*Lunch*
14:15-16:00	**Symposium 8 – Gene editing and delivery**
	Chairs: Mitch Drumm (US) / -
14:15-14:40	Systemic in utero gene editing as a treatment for cystic fibrosis - Marie E. Egan (US)
14:40-15:05	Artificial intelligence-guided design of lipid nanoparticles for pulmonary gene therapy - Jacob Witten (US)
15:05-15:30	Development of mRNA and ribonucleoprotein delivery tools for lung targeted gene therapy in cystic fibrosis - Anna Cereseto (IT)
15:30-15:40	Abs. 74 - Journey to the Centre of the Airway: tackling cystic fibrosis with cell-penetrating VP22-ABEs - Joana Alves (IE)
15:40-15:50	Abs. 67 - Development of a novel Adenine Base Editor with temporospatial control of the editing - Immacolata Zollo (PT)
15:50-16:00	Abs 69 - Precise correction of challenging mutations causing cystic fibrosis through transient delivery of the prime editing machinery - Laudonia Lidia Dipalo (BE)
16:00-16:30	*Coffee Break*
16:30-17:30	**Closing Keynote lecture** Advancing therapies for cystic fibrosis in an evolving global landscape - Deepika Polineni (US)
20:00	*Dinner / Social Event*

POSTER TITLES & AUTHORS

P1 DNA Methylation of Long Interspersed Nuclear Element 1 (LINE-1) in nasal epithelial cells from Georgian cystic fibrosis patients with rare c.1545_1546delTA pathogenic variant
E. Kvaratskhelia, S. Surmava, M. Gagua, E. Maisuradze, L. Livshits, K. Kankava, E. Abzianidze, T. Tkemaladze

P2 Specific stages of iPSC to airway basal cell differentiation exhibit variable organoid swelling in response to CFTR modulators in a cystic fibrosis rare mutation model
X.J. Teoh, B. Cavanagh, A. Berical, F. Hawkins, D. Kotton, P. McNally, K. Hurley, I. Oglesby

P3 Antisense oligonucleotide amenability of splicing and nonsense mutations in cystic fibrosis
M.D Leahy, L.A Clarke , M.D Amaral

P4 Novel RNA binding proteins regulate the levels of PTC-containing CFTR mRNA
A. Almeida Henriques, A. Abrantes, C.M Farinha

P5 Optimization of blocking oligonucleotides as therapies to correct consequences of deep intronic mutations
C. Felgerolle, K. Deletang, M. Taulan-Cadars

P6 Nonsense suppression of CFTR PTC variants through attenuation of translation termination and mTOR inhibition
F. Liang, H. Shang, A. Pande , S. Unger , Y. Cheng , S. Cantu , L. Wang , K. Coote , H. Bihler , M. Mense

P7 Novel readthrough compounds for the treatment of cystic fibrosis patients with nonsense mutations
I. Pranke, A. Hatton, M. Djumagulov, E. Dreano, M. Kelly, A. Hinzpeter, L. Bidou, O. Namy, J.-P. Renaud, I. Sermet-Gaudelus

P8 Developing novel ribosome-directed small molecules and antisense oligonucleotides as therapeutic approaches for refractory CFTR variants
J.J. Jackson , C. Foye, A.G. Winters , E. Freestone, D. Patel, Y. Du, L. Huang, K.E. Oliver

P9 Inflammatory stimuli enhance pharmacological rescue of CFTR nonsense mutations
A. Borrelli, A. Venturini, D. Guidone, M. De Santis, F. Ciciriello , L.J.V. Galletta

P10 Identification of novel pharmacological modulators of the NMD mechanism to recover CFTR function in patients with nonsense mutations
A. Venturini, A. Borrelli, F. Ciciriello, L.J. Galietta

P11 The cereblon E3 ligase modulator, CC-90009, rescue CFTR nonsense variants promoting NMD suppression and PTCs readthrough in rectal organoids
R.V. Latorre, C. Mortali, D. Scutelnic, M.M. Bogdan, K. Kleinfelder, M.M. Mina, A. Massella, F. Tomba, C. Daffara, P. Melotti, C. Sorio

P12 Peptide-based CFTR stabilizers as novel therapeutic tools in cystic fibrosis
C. TEKO-AGBO, M. SINANE, K. DELETANG, C. FELGEROLLE, M. TAULAN-CADARS, P. BOISGUERIN

P13 CFTR stabilizers as therapeutic tools in cystic fibrosis
M. Sinane, K.Deletang, C. Felgerolle, C. Teko-Agbo, P. Boisguerin, M. Taulan-Cadars

P14 Partial inhibition of proteins in distinct structural regions of the ribosome confer differential rescue of N- versus C-terminal CFTR nonsense variants
A.G. Winters, E. Freestone, W. Wang, M.H. Ali, J.J. Jackson, C. Foye, J.L. Hartman IV, E.J. Sorscher, K.E. Oliver

P15 Searching for Small Molecule Modulators of Premature Termination Codon Variants in the CFTR Gene
L. Clarke, M. Leahy, H. Botelho, V. Cachatra, C. Moiteiro, M. Amaral

P16 Investigating the therapeutic effects of K+ channel modulators on mucus and airway surface liquid in cystic fibrosis
O. Hamed, Z. Chen, D.C.H. Benton, V. Dua, G.W. Moss

P17 An Investigation of The Importance of pH in Controlling Mucin Rheology
S. Li , D.C.H. Benton, G.W.J. Moss

P18 The apical mucus layer alters the pharmacological properties of the airway epithelium
D. Guidone, M. De Santis, E. Pesce, V. Capurro, N. Pedemonte, L.J. Galietta

P19 *Ex vivo* pig lung as a new Cystic Fibrosis model for the study of *Pseudomonas aeruginosa* biofilm infection and phage therapy application
M. Cafora, F. Forti, V. Capurro, F. Harrison, F. Briani, A. Pistocchi

P20 Utilisation of human induced pluripotent stem cells for modelling cystic fibrosis lung disease *in vitro*
M.-C. Jaboreck, A. Balázs, J. Zöllner, N. Cleve, L. Czichon, L. von Schledorn, J. Hegermann, J.C. Nawroth, D. Roth, M. Mielenz, S. Hedtfeld , F. Stanke, T. Rubil, F. Ius, D. Jonigk, J.W. Hanrahan, R. Olmer, M.A. Mall, S. Merkert, U. Martin

P21 Development of new models to study the impact of CFTR dysfunction on osteocytes in cystic fibrosis-related bone disease
L. Thoraval , C. Guillaume, L. Hamon, G. Bouet-Chalon, A. Vanden-Bossche, D. Marchat, F. Velard

P22 Pancreatic cancer growth rate may be susceptible to variations in CFTR expression
R. Ibáñez, J.P. Muñoz, M. Corbacho, J. Barquinero, M.T. Salcedo, L. Campderros, E.C. Vaquero, X. Molero

P23 Does CFTR Play a Role in Epithelial Differentiation, EMT and Cancer?
C.S Rodrigues, I. Pankonien , V. Railean, M.D Amaral

P24 Key role of bone marrow adiposity in the development of Cystic Fibrosis-related Bone Disease
L. Hamon, C. Dumortier, L. Thoraval, J. Sergheraert, O. Piot, E. Buache, N. Goffin, C. Chauveau, S. Gangloff, D. Al Alam, M.-L. Jourdain, F. Velard

P25 Elexacaftor/tezacaftor/ivacaftor CFTR modulators mitigate senescence in cystic fibrosis
A.M. Hristodor, T. Gunawardena, M. Borgatti, A. Vella, C. Boni, D. Olioso, F. Quiri, G. Lippi, T. Moraes, M. Cipolli, V. Bezzerri

P26 Selective impairment of CFTR-associated bicarbonate transport is recovered by CFTR modulators in vitro in an individual carrying the F508del/L997F CFTR genotype affected by recurrent pancreatitis
K. Kleinfelder, U. Krivec, P. Pascolo, A.P. Novak, F. Amato, A.M. Hristodor, M. Bertini, E. Pintani, P. Melotti, C. Sorio

P27 Characterization of a frameshift variant by means of patient-derived nasal epithelial cells: molecular and functional analysis of *CFTR* mRNA and protein
C. Pastorino, V. Capurro, V. Tomati, E. Pesce, M. Lena, A. Dighero, L. Menta, R. Bocciardi, N. Pedemonte

P28 Investigating the safety of Trikafta/Kaftrio exposure during early development
A. Squarzoni, S.M. Bertozzi, M. Summa, R. Bertorelli, T. Bandiera, G. Capodivento, C. Montani, V. Tomati, V. Capurro, N. Pedemonte, F. Benfenati, L. Nobbio, A. Armirotti

P29 GoSlo, a Potassium Channel Modulator, Enhances CFTR Activity in Wild-Type and F508del-CFTR Variants
J.N. Lunavath, G.W.J. Moss, P. Vergani

P30 In silico, in vitro and ex vivo characterization of CFTR pathogenic variants localized in the Fourth Intracellular Loop and their rescue by modulators
E. Pesce , V. Tomati, V. Capurro, M. Lena, C. Pastorino, M. Astore, S. Kuyyucak, B. Chevalier, E. Sondo, F. Cresta, V. Terlizzi, S. Costa, M.C. Lucanto, V. Daccò, L. Claut, F. Ficili, R. Bocciardi, F. Zara, C. Castellani, L.J. Galietta, S.A. Waters, A. Hinzpeter, N. Pedemonte

P31 Deleterious effect of *P. aeruginosa* on F508del-CFTR rescued by Elexacaftor/Tezacaftor/Ivacaftor is clinical-strain dependent in patient-derived nasal cells
C. Allegretta, E. Montemitro, M.N. Sgobba, V. Capurro, E. Pesce, F. Ciciriello, G. La Bella, M. Rossito, V. Tuccio, F. Arena, T.N. Gunawardena, L. Guerra, N. Pedemonte, N. Capitanio, C. Piccoli, O. Laselva

P32 Structure-guided combination of novel CFTR correctors to improve the function of F508del-CFTR in airway epithelial cells
C. Allegretta, D. Lunaccio, N. Scarano, V. Vinci, A. Salis, B. Tasso, C. Brullo, N. Capitanio, C. Piccoli, E. Cichero, E. Millo, O. Laselva

P33 The inverted formin INF2 in the regulation of CFTR expression and trafficking
J.F Ferreira, C. Alves, N. Pedemonte, V. Tomati, C.M Farinha

P34 Novel class 3 CFTR correctors suitable for combinatorial treatments in cystic fibrosis
M. Barreca, F. Lo Mascolo, A. Lipani, S. Giuffrida, V. Spanò, M.V. Raimondi, A. Montalbano, P. Barraja, M. Renda, A. Borrelli, A. Venturini, D. Guidone, M. Genovese, L.J.V. Galietta, F. Bertozzi, T. Bandiera

P35 Primary Nasal Epithelial Cells for Personalized Medicine in non-eligible Cystic Fibrosis Patients
J. Berger, A. Balász, T. Rubil, A. Gonzáles, J. Berges, Y. Yun, O. Sommerburg, M. Stahl, M.A. Mall, S.Y. Graeber

P36 Nitrate enhances the activity of F508del-CFTR channels rescued by elexacaftor-tezacaftor-ivacaftor
J.N. Charlick , D.N. Sheppard

P37 CFTR expression and function in human foetal pancreatic ducts and ductal organoids
L. Kiss, G. Mihalekné Fűr, Á. Dágó, E.M. Orján, Z. Balla, E.R. Bálint, E.S. Kormányos, P. Pallagi, N. Pásztor, L. Kaizer, V. Venglovecz, J. Maléth, P. Hegyi, Z. Kozinszky, Z. Rakonczay Jr.

P38 Clinically approved β2 agonist formoterol enhances CFTR modulator rescue of N1303K-CFTR via a dual mode of action
M.M Ensinck, A.S. Ramalho, E. Bulik-Sullivan, M. Nijs, S. Van Belle, S. Thierie, L. De Keersmaecker, S. Munck, H. Klaassen, F. Christ, Z. Debyser, A. Hinzpeter, I. Sermet-Gaudelus, C.M. Ribeiro, S.H. Randell, F. Vermeulen, M.S. Carlon

P40 Inflammation modulation using anti-inflammatory protein Tristetraprolin axed therapies
T. Dumas, S. Bleuse, K. Deletang, P. Boisguerin, A. Bourdin, M. Taulan-Cadars

P41 Utilizing artificial intelligence and robotization to research the entire dynamic range of CFTR function in CF patient-derived intestinal organoids
S. Kroes , S. Spelier, B. Bosch, L. Winkel, S. Beuningen, J. Beekman

P42 Mapping the Impact of ETI-mediated CFTR Reactivation On Development: a zebrafish model study
M. Cafora, S. Pagliughi, D. Dobi, G. Galassi, A. Mantero, M. Aureli, A. Pistocchi

P43 Enhancing F508del-CFTR Plasma Membrane Stability: Lipid and Modulator Synergies
D. Dobi, N. Loberto, A. Tamanini, R. Bassi, L. Mauri, D. Olioso, N. Pedemonte, M. Aureli

P44 A Little Peptide for a Smooth Ride: Enhancing ETI-Mediated F508del-CFTR Stabilization with a PI3Kγ Mimetic
M. Mergiotti, A. Murabito, V. Capurro, A. Loffreda, P. Peretto, M. Li, K. Ren, A. Raimondi, C. Tacchetti, D. Diviani, N. Pedemonte, E. Hirsch, A. Ghigo

P45 ATP-dependent modulation of the NBD1 conformational equilibrium from CFTR channel
A. Svoeglazova, M. Overtus, T. Kache, C. Martens, J. Hendrix, C. Govaerts

P46 Beyond chloride: a multi-omic integration analysis to identify the effects of Elexacaftor/Tezacaftor/Ivacaftor in F508del primary airway epithelial cultures
M. Kelly-Aubert, V. Laigle, E. Bardin, A. Hatton, C. Guerrera, C. Bole, S. Grassin Delyle, V. Stoven, I. Sermet-Gaudelus

P47 Theranostics for People with Cystic Fibrosis: A561E-CFTR is rescued by elexacaftor/tezacaftor/ivacaftor
C.S Rodrigues, I. Pankonien, V. Railea, I. Coelho, M.D Amaral

P48 Proteases overactivation in predisposing the cystic fibrosis airway epithelium to infections
P. Bigot, M. Badaoui, R. Vivès, M. Chanson

P49 Iterative *in silico* and *in vitro* screens identify compounds simultaneously improving CFTR channel function and biogenesis
M.-C. Ardelean, S. Javor, J.-L. Reymond, P. Vergani

P50 CFTR activity in nasal epithelia from subjects with different genotypes
V. Capurro, E. Pesce, V. Tomati, C. Pastorino, M. Lena, A. Dighero, L.J.V. Galietta, F. Cresta, C. Castellani, N. Pedemonte

P51 Optimizing scalable nasal airway organoid generation for automated high-throughput screening
A. Shekhar, L. Winkel, S. Beuningen, G.Amatngalim, J. Beekman

P52 Rescue of p.Phe508del-CFTR through inhibition of the kinase GRK5: identification of proteins and small molecule mediators
M.F. Caleiro, D. Neugebauer, S. Abdul, I. Michou, R. Pacheco, M.D. Amaral, H.M. Botelho

P53 Study of potential therapeutic approaches for cystic fibrosis patients with rare mutations in Sri Lanka
T. Gunawardena, S. Kay, G. Langeveld, A. K.W.D. Anuradha, E. Jasinghe, F. Ratjen, M. Solomon, V. Dissanayake, C. E. Bear

P54 A novel active conformation of CFTR may drive functional recovery of F508del-mutant
C. Govaerts, M. Overtus, T. Rubil, B. Loughlin, Z. Rich, M. Rodrat, Z. Yang, A. Balazs, J.C. Kappes, M. Mall, D.N. Sheppard, J.F. Hunt

P55 Synergy between stabilizing nanobody and approved correctors enables remarkable functional recovery of F508del-CFTR
M. Overtus, T. Rubil, J.N. Charlick, M. Rodrat, A. Balázs, M. Mall, D.N. Sheppard, C. Govaerts

P57 Esc peptides enhance CFTR gating and combat *Pseudomonas aeruginosa*
M.L. Mangoni, L. Ferrera, M. Mori

P58 Unveiling new insights of ionocytes' role in cystic fibrosis using single-cell RNA sequencing analysis and human induced pluripotent stem cell-derived airway epithelial cells
M. Salvà-Barceló, J. Muncunill-Farreny, L. Vallier, J.M. Ramis, M. Monjo, M. Vilà-González

P59 GY971, a new anti-inflammatory agent for the treatment of Cystic Fibrosis lung disease
I. Lampronti, C. Tupini, S. Fagnani, G. Raso, A. Chilin, G. Marzaro, A. Tamanini, N. Pedemonte, V. Capurro, O. Laselva, C. Allegretta, A. Bragonzi, G. Cabrini

P60 Hyaluronic acid derivates as new potential anti-inflammatory agents for Cystic Fibrosis lung disease
I. Lampronti, S. Fagnani, C. Tupini, G. Raso, G. Cabrini, S. Manfredini, A. Baldisserotto

P61 Mitochondrial metabolic rewiring drives wound healing in cystic fibrosis primary airway epithelium cultures treated with Elexacaftor/Tezacaftor/Ivacaftor
M. Kelly-Aubert, E. Bardin, V. Laigle, A. Deslys, A. Hatton, B. Chevalier, I. Kühl, A. Imbard, E. Le Guillou, P. Vargas, V. Stoven, I. Sermet-Gaudelus

P62 Evaluation of amniotic epithelial cells (hAECs) as a cell-based therapeutic approach for cystic fibrosis
M. Lena, V. Tomati, V. Capurro, E. Pesce, C. Pastorino, A. Dighero, R. Gramignoli, N. Pedemonte

P63 Modeling a Triple Culture Airway-on-a-Chip for Inflammation Studies in Cystic Fibrosis
Y. Man, A. Jiang, A. Stejskalová, H. Bai, G. Di Bonaventura, M. Romano, D. Ingber, R. Plebani

P64 Gut microbiota-metabolome relationships across pwCF taking highly effective CFTR modulators: Preliminary findings from the GRAMPUS-CF study
R. Marsh, W. Cheung, D. Arends, D. Sills, D.W Rivett, A.R Smyth, C. van der Gast

P65 The gut's role in pulmonary and extrapulmonary manifestation of Cystic Fibrosis
C. Cigana, G. Delmonte, A. Facoetti, L. Veschetti, F. Gianferro, A. Neri, R. Fiorotto, A. Livraghi-Butrico, L. Massimino, F. Ungaro, A. Bragonzi

P67 Development of a novel Adenine Base Editor with temporospatial control of the editing
I. Zollo, L. Santos, J. Alves, A.S Coroadinha, P.T Harrison, C.M Farinha

P68 Correction of F508del via Interhomolog Homologous Recombination in compound heterozygote cell lines
L. Lonetti, J.B. Murray, C.M. Lee, P.T. Harrison

P69 Precise correction of challenging mutations causing cystic fibrosis through transient delivery of the prime editing machinery
M. Bulcaen, L.L. Dipalo, B. Poelmans, S. Thierie, M. Smits, M. De Roy, M. Ensinck, A. Ramalho, Z. Debyser, F. Vermeulen, R. Gijsbers, M. Carlon

P70 Whole Exon Correction Approach for CF: Repair of All CF-causing Variants in Exon 12 with Homology-Directed Repair
E. Collins, J. Murray, J. Alves, P. Harrison

P72 Humanized CFTR exon-replacement mice to accelerate development of nucleic acid-directed therapies
R. Conlon, C. Hodges

P73 A split and inducible precise adenine base editor for a less promiscuous correction of W1282X
J. Alves, L. Santos, C. M Farinha, P. T Harrison

P74 Journey to the Centre of the Airway: tackling cystic fibrosis with cell-penetrating VP22-ABEs
J. Alves, C. M. Farinha, P. T. Harrison

P75 Development of GE-vesicles as CRISPR-Cas delivery system in genome editing applications for cystic fibrosis
G. Maule, M. Stancampiano, E. Gurrieri, F. Zerbini , A. Cereseto

P76 Predicting benchmark efficiency for CF gene therapies using 3D analyses of individual mosaic organoids
J. Bulkens, A. Griffioen, N. Ithaksiou, M. Smith, S. van Beuningen, J. Beekman, E. Kuijk

P77 A multicellular modelling framework for airway epithelial fluid and ion transport: implications for cystic fibrosis gene therapy and building towards a functional atlas
O. Hamed, G.W. Mos, V. Dua

P78 A novel LNP formulation for the targeted delivery of gene editing tools to lung epithelial cells
S. Saxena, S. Hak, A. Colliva, S. Vodret, J. Parot, A. Molska, G. Maule, E. Guerrieri, M. Stancampiano, S.E. Borgos, A. Cereseto, S. Zacchigna

AWARD WINNERS

ECFS Young Fellows Travel Award

Anna Borrelli (IT)
James Charlick (UK)
Theo Dumas (FR)
Marjolein Ensinck (BE)
Omar Hamed (UK)
JaNise Jackson (US)
Sharad Saxena (IT)
Immacolata Zollo (PT)

Student Helper Award

Joana Alves (IE)
Laudonia Dipalo (BE)
Mark Leahy (PT)
Miriam Salvà-Barceló (ES)

Free Registration Young Researchers

Supported by the Italian Cystic Fibrosis Research Foundation

Caterina Allegretta
Marco Cafora
Marco Mergiotti
Cristina Pastorino
Angelica Squarzoni
Arianna Venturini

26 March — 18:00–19:00

Opening Keynote Lecture

CFTR, from folding to function

Ineke Braakman, Peter van der Sluijs

Cellular Protein Chemistry, Science4Life, Faculty of Science,Utrecht University, Utrecht, Netherlands

Protein function requires proper protein structure, which is why mutations in the CFTR protein that affect its function cause disease.

To restore CFTR functional defects, small-molecule medicines have been developed that act directly on the CFTR proteins. Potentiators act on CFTR structure and function directly by acutely opening the chloride channel upon addition. Correctors however need to work earlier, while CFTR is acquiring its structure, while it is folding.

The development of these modulators, which correct or boost CFTR function has been preceded by an understanding of the correctability of F508del CFTR, and has been accompanied by deep insights into the folding of wild-type and misfolding of disease-causing CFTR mutants.

With this knowledge and available assays, the mode of action of modulators could be established to such an extent that we now understand how defective CFTR can be rescued. This is an unprecedented advance for any disease field, to be credited to funders and scientists alike.

27 March — 08:45–10:30

Symposium 01 - Therapeutic approaches for Nonsense mutations

S1.1 Fighting the nonsense. From basic science to new drugs

I Sermet-Gaudelus[1], Alexandre Hinzpeter[1], O Namy[2]

[1] INSERM U1151, Institut Necker Enfants Malades, Paris, France, [2] Institute for Integrative Biology of the Cell. Saclay, France

Nonsense mutations originate from single nucleotide substitutions introducing an in-frame Premature Termination Codon (PTC), TAA, TAG or TGA in the Open Reading Frame. They reduce mRNA stability which induces the elimination of the PTC-containing transcripts by the nonsense-mediated decay (NMD) pathway and when the deficient mRNA escapes NMD, they induce the production of a truncated protein, most of the time non-functional and toxic, targeted for elimination.

Currently there are no therapeutic options for people with CF (pwCF) carrying nonsense mutations. Gene therapy, DNA-based therapy by CRISPR/Cas9, RNA-based therapies by messenger RNA (mRNA) or transfer RNA (tRNA) are currently being tested, aiming for topical administration, but must overcome natural barriers like airway mucus, phagocytes uptake, entrance into highly differentiated cells with a low dividing rate and rapid degradation of RNA.

There has been very active pharmacological research for novel effective drugs promoting translational readthrough of PTCs. This favors the recruitment of a near-cognate tRNA to outcompete the binding of the translation termination factor eukaryotic release factor 1 (eRF1) into the ribosomal A-site, allowing the translation elongation to continue in the correct reading frame.

At the molecular level, Aminoglycosides (AGs), such as G418 (geneticin), gentamicin, bind to the decoding center of the ribosome and enhance accommodation of near-cognate tRNA at A-site bound PTCs and favor translation misreading. Based on a "ligand-based" approach, less toxic and more efficient AG derivatives were developed such as NB124, ELX-02, which proved efficient *in vitro* for W1282X, G542X, and rare PTCs. However, ELX-02 phase 2 clinical trial in pwCF carrying the G542X variant led to disappointing results. Other drug candidates were detected by high-throughput screening, including ataluren, clitocine, escin, and, more recently 2,6-diaminopurine, efficient for UGA PTCs, and SRI-41,315 which reduces the abundance of the termination factor eRF1 and induces a prolonged pause at the PTC.

Multiple challenges remain to achieve efficient readthrough. First, the abundance of the transcript available for translation and subsequent readthrough is reduced by the NMD pathway. Second, for the PTC-containing transcripts that reach the ribosome, the PTC surrounding context affects the readthrough level, very probably due to the local sequence, as well as the availability of specific tRNA in the vicinity. As a consequence, most of the time, none or only a fraction of the full length "neo-protein" is native while the others may not be functional. Most importantly the readthrough level is very low and the drugs developed so far perform poorly apart from AGs which are, in fact, too toxic to be administered lifelong.

These barriers have led our consortium to investigate novel approaches for more potent readthrough compounds identification which will be presented. TLN468 result in the specific stimulation of PTC readthrough, with no alteration of normal termination process. A "structure-based drug design" funded by high-resolution X-ray and cryo-EM structures of the full translation module composed of the eukaryotic ribosome in complex with mRNA, tRNA, and the elongation factor eEF-2 enabled identification of new compounds, whose efficiency on different CFTR-PTCs is currently ongoing.

Leroy C, et al, PMID: 36641622. Bidou L, et al, PMID: 35994666. Milicevic N, et al, PMID: 38030725.

S1.2 Premature stop-codon readthrough by R-ASO

Andrei Korostelev

RNA Therapeutics Institute, University of Massachusetts Chan Medical School, Worcester, MA, USA

Premature nonsense mutations remain a leading cause of cystic fibrosis. A nonsense mutation results in the expression of a truncated CFTR protein, and therapeutic strategies aim to restore full-length protein expression. Most strategies under development, including small-molecule aminoglycosides, suppressor tRNAs, or the targeted degradation of termination factors, lack mRNA target selectivity and may poorly differentiate between nonsense and normal stop codons, resulting in off-target translation errors. We demonstrate that antisense oligonucleotides can stimulate readthrough of disease-causing nonsense codons, resulting in high yields of full-length protein in mammalian cellular lysates. Readthrough efficiency depends on the sequence context near the stop codon and on the precise targeting position of an oligonucleotide, whose interaction with mRNA inhibits peptide release to promote readthrough. Readthrough-inducing antisense oligonucleotides (R-ASOs) enhance the potency of non-specific readthrough agents, including aminoglycoside G418 and suppressor tRNA, enabling a path toward target-specific readthrough of nonsense mutations in *CFTR* and other mutant genes. Finally, through systematic chemical engineering, we identify heavily modified fully functional R-ASO variants, enabling future therapeutic development.

S1.3 Development of a platform therapeutic for nonsense-associated cystic fibrosis

Joseph J. Porter[1], Emily G. Sorensen[1], David A. Dean[2], John D. Lueck[1,2,3]

[1]Department of Pharmacology and Physiology, University of Rochester School of Medicine and Dentistry, Rochester, NY 14642, USA., [2]Department of Neurology, University of Rochester School of Medicine and Dentistry, Rochester, Rochester, NY 14642, USA., [3]Center for RNA Biology, University of Rochester School of Medicine and Dentistry, Rochester, Rochester, NY 14642, USA.

Nonsense mutations or premature termination codons (PTCs) occur when a canonical triplet nucleotide codon is converted into one of three stop codons (TGA, TAG and TAA). These mutations make up 10-15% of all genetic lesions that cause disease including cystic fibrosis. We have recently demonstrated the function of a series of nonsense suppressor anticodon edited tRNAs (ACE-tRNAs) to suppress the most common CF-causing PTCs in the cystic fibrosis transmembrane regulator (*CFTR*) gene, accounting for ~70% of people with nonsense-associated CF. A major advantage of ACE-tRNAs as a therapeutic cargo is the small (~125 bp) size of the ACE-tRNA gene including promoter and transcriptional terminator. Taking advantage of this property, we have previously encoded ACE-tRNAs in non-viral DNA minivectors, which have several favorable characteristics that include increased cell transfection efficiency and higher molar equivalents of the cargo sequence per vector delivered. Through these efforts, we now understand that linear covalently closed DNA vectors with sizes as small as 200 bp can serve to support significant PTC suppression when electroporated or transfected into cultured cells. However, this approach towards development of a therapeutic cargo for treatment of PTCs has technical challenges related to minivector production methods, which exhibited poor scalability, adaptability, and GMP adherence. With this in mind, we developed a novel assembly method for production of synthetic linear ACE-tRNA picovectors (sLPV; 'picovectors' owing to their small size) using substrates produced by solid-phase oligonucleotide synthesis, with DNA synthesized to contain internal DBCO functionalized nucleotides for copper-free click labeling with functionalized moieties. Introduction of this chemical functionality allows for dual labeling of the sLPVs with any combination of fluorophores, peptides, proteins, or other DNAs to improve cellular delivery, intracellular trafficking, and affords intracellular imaging of the sLPVs. Using this approach we labeled ACE-tRNA picovectors with peptides comprising several different nuclear localization signals (NLS) for active transport to the nucleus in non-dividing cells. This novel modular sLPV assembly procedure allows for production of minimal ACE-tRNA therapeutic vectors with properties that can be engineered for optimal cellular delivery and intracellular trafficking to the airway of lungs or other target tissues.

P10

S1.4 Identification of novel pharmacological modulators of the NMD mechanism to recover CFTR function in patients with nonsense mutations

A. Venturini[1], A. Borrelli[1], F. Ciciriello[2], L.J. Galietta[1]

[1]TIGEM - Telethon Institute of Genetics and Medicine, Pozzuoli, Italy, [2]Cystic Fibrosis Center, "Bambino Gesù" Children's Hospital, IRCCS, Rome, Italy

Background: Nearly 10% of CFTR mutations, particularly premature termination codons (PTCs), are insensitive to CFTR modulators (i.e. correctors and potentiators). The pharmacological rescue of PTCs requires a combinatorial approach. First, compounds that inhibit the nonsense-mediated RNA decay (NMD) mechanism are crucial to increase the amount of CFTR transcripts. In addition, readthrough agents are required to allow the continuation of protein synthesis, by inserting an amino acid in the site of PTC. NMD inhibitors can also be effective in the absence of a readthrough agent for PTCs localized at the carboxy-terminus of the CFTR sequence such as W1282X. The aim of this project was to identify novel molecules able to modulate the NMD mechanism.
Methods: We used the 16HBE14o$^-$ cell line expressing W1282X-CFTR (obtained from CFF) and stably transfected with the halide-sensitive yellow fluorescent protein (HS-YFP) to screen a chemical library of more than 9,000 compounds, including drugs, investigational drugs, and compounds with known biological activity (mechanistic probes). The most effective compounds have then been characterized by secondary biochemical and functional assays to understand their mechanism of action.
Results: We have completed the screening by the HS-YFP functional assay, and we identified 75 hits. Molecules showing the greater effect on CFTR rescue were further tested in the presence/absence of CFTRinh-172 to confirm a direct activity on CFTR channel and not on other anion channels/transporters. We focused our attention on three different compounds: NMDi-01, NMDi-02, NMDi-03. We evaluated their effects both at the transcriptional and protein expression level. These compounds induced more than 10-fold increase in CFTR-mRNA transcripts and promoted the appearance of a signal for CFTR protein. We then tested their efficacy on W1282X mutation at the functional level, by conducting short-circuit current recordings experiments. NMDi-01 and NMDi-02, in combination with CFTR correctors, were able to induce a marked increase in CFTR channel activity. Interestingly, NMDi-03 *per se* elicited a significant increase in CFTR function, that was further amplified when combined with correctors. To obtain further information about the mechanism of action of active compounds, we performed an analysis of their effect on 16HBE14o- cell transcriptome, both the wild type and W1282X versions. Preliminary results show that CFTR transcript is specifically upregulated in W1282X cells, in agreement with NMD specifically affecting the CFTR mRNA with PTC. A deep bioinformatic analysis is being carried out to analyze the data obtained by RNA seq. The goal is to highlight common and compound-specific signatures elicited by treatments.
Conclusions: We have identified three possible candidates acting as NMD inhibitors/modulators that could increase CFTR rescue in patients with PTCs.
Acknowledgements: This project is supported by CFF (GALIET22I0), the European Cystic Fibrosis Society and CF Europe (post-doctoral research fellowship to Arianna Venturini).

P8

S1.5 Developing novel ribosome-directed small molecules and antisense oligonucleotides as therapeutic approaches for refractory CFTR variants

J.J. Jackson [1,2], C. Foye[1,2], A.G. Winters [1,2], E. Freestone[1,2], D. Patel[1,3], Y. Du[4,5], L. Huang[6], K.E. Oliver[1,2]

[1]Department of Pediatrics, Emory University School of Medicine, Atlanta, United States, [2]Center for Cystic Fibrosis and Airways Disease Research, Children's Healthcare of Atlanta and Emory University School of Medicine, Atlanta, United States, [3]Center for ViroScience and Cure, Laboratory of Biochemical Pharmacology, Children's Healthcare of Atlanta and Emory University School of Medicine, Atlanta, United States, [4]Department of Pharmacology and Chemical Biology, Emory University School of Medicine, Atlanta, United States, [5]Emory Chemical Biology Discovery Center, Emory University School of Medicine, Atlanta, United States, [6]Ionis Pharmaceuticals, Carlsbad, United States

Background: Of the 2,121 *CFTR* variants reported to date, 271 are on-label for clinically approved CFTR modulators in the United States. *CFTR* variants that have demonstrated insensitivity to these compounds, and/or have not been tested for drug responsiveness, largely encompass severe defects in CFTR synthesis (e.g. Class I variants) and processing (e.g. Class II variants). Among Class I variants, nonsense alleles are particularly difficult to overcome due to premature degradation of the encoded mRNA and protein. Our work endeavors to identify and therapeutically target genetic interactions that influence biogenesis of these and other refractory *CFTR* variants. We previously discovered ribosomal protein L12 (RPL12/uL11) as a robust modifier of mutant CFTR processing, with ~50% knockdown of RPL12 conferring improved functional expression of specific variants from different *CFTR* subclasses (e.g. F508del, W1282X). In the present study, novel antisense oligonucleotides (ASOs) and small molecule inhibitors of RPL12 were developed and evaluated for potential to rescue the same *CFTR* variants.

Methods: ASOs targeting human RPL12 were engineered by Ionis Pharmaceuticals. These molecules were tested for efficacy in CF bronchial epithelia (CFBE41o-) and primary human nasal epithelia (HNE) encoding wild-type or F508del-CFTR. For high-throughput drug screening (HTS), Fischer rat thyroid (FRT) cells were stably transduced with human RPL12 or W1282X-CFTR encoding a C-terminal, in-frame NanoLuc reporter. Measures of cell viability (Promega CellTiter-Fluor Assay), RPL12 or CFTR luciferase signal (Promega NanoGlo Assay), CFTR mRNA expression (qRT-PCR), protein maturation (western blot), and/or channel function (Ussing chamber) were employed. Studies included treatment comparisons to negative controls (scrambled ASO, empty vehicle) and positive controls such as established inhibitors of translation (G418, PTC-124, ELX-02, Escin).

Results: In CFBE and HNE, we show two ASOs decrease RPL12 transcripts and protein to similar levels achieved with siRNAs. These RPL12 ASOs significantly augment wild-type and F508del-CFTR band C maturation, in addition to F508del-dependent short-circuit currents. In FRT cells, early HTS results revealed 47 compounds at which ~50% suppression of RPL12 is attained. These hits are presently undergoing: (1) dose-response confirmatory screens; (2) counter-screens with cell viability to exclude compounds demonstrating toxicity; (3) counter-screens against NanoLuc protein alone as a measure of specificity; (4) application to FRT cells expressing W1282X::NanoLuc to evaluate readthrough efficiency; and (5) structure-activity relationship assessments to optimize functional group modifications that correlate with improved RPL12 suppression and mutant CFTR rescue.

Conclusions: Partial depletion of RPL12 levels represents a feasible strategy for CFTR modulation, which may be applicable to *CFTR* genotypes refractory to available clinical interventions. Our work serves as a foundation from which future investigations may be pursued to examine efficacy and tolerability of anti-RPL12 compounds or ASOs delivered to CF animal models. This study was supported by the NIH, U.S. CFF, and Atlanta Pediatric Research Alliance.

P6

S1.6 Nonsense suppression of CFTR PTC variants through attenuation of translation termination and mTOR inhibition

F. Liang, H. Shang, A. Pande , S. Unger , Y. Cheng , S. Cantu , L. Wang , K. Coote , H. Bihler , M. Mense

Cystic Fibrosis Foundation, CFFT Lab, Lexington, United States

Background: Nonsense suppression is a natural biological process where ribosomal translation occasionally occurs at a premature termination codon (PTC) or nonsense mutation in mRNA. Despite significant efforts to develop therapeutic modulators to stimulate PTC readthrough, the biochemical and cellular processes underlying ribosomal readthrough modulation remain largely unknown.

Ribosome-binding antibiotics, such as Geneticin (G418), have been shown to facilitate translational readthrough of PTC mutations in multiple mRNAs, including those responsible for disease-causing CFTR variants. High throughput screening (HTS) aimed at identifying readthrough modulators has revealed chemical modulators with diverse structures, most notably, ribosome-binding antibiotics and degraders of the eucaryotic release factor 1 (eRF1) [1]. Biological profiling of readthrough modulating agents aims to identify targets and better understand the biological mechanisms of translational readthrough. Here, we evaluate the effects of two classes of readthrough modulators on nonsense mutation suppression: eRF modulators and mTOR inhibitors.

Results: Translational readthrough of native CFTR R1162X was observed when CFF 16HBEge CFTR R1162X cells were treated with CC-90009, a degrader of the translation termination factor eRF3a. Combining aminoglycosides (G418, ELX-02) with CC-90009 resulted in the readthrough of native CFTR R1162X, achieving significant levels of full-length CFTR protein equivalent to 20% of the normal CFTR expression in parental 16HBE14o- cells. Additionally, the functional restoration of CFTR R1162X by ELX-02 in the TECC-24 assay was significantly (~3-fold) enhanced in the presence of CC-90009.

HTS of the ReFrame drug repurposing library at the CFFT Lab identified six known, chemical diverse mTOR inhibitors. Two commercially available analogs of one of the mTOR inhibitor hits were also tested and confirmed in the HTS assay. Preliminary results suggest that the hit compounds with mTOR inhibitor activity suppress nonsense-mediated decay (NMD) and ribotoxic stress response (RSR).

Conclusion: Modulator screens have validated ribosome-binding antibiotics and eRF modulators for readthrough and have revealed additional mechanisms. A better understanding of the biological processes of nonsense suppression is crucial for developing therapeutic strategies to manage diseases caused by PTCs. For example, understanding the signaling/mechanisms of mTOR inhibitors may allow the identification of a more specific target for readthrough in CF.

[1] Sharma J et al. A small molecule that induces translational readthrough of CFTR nonsense mutations by eRF1 depletion. Nat Commun. 2021;12:4358. http://doi.org/10.1038/s41467-021-24575-x.

27 March — 11:00–12:45

Symposium 02 - Mucus and mucins in CF

S2.1 Mucins under pressure: the key to understanding mucus stasis in CF

Cameron Morrison[1,2,3,4], Kendall ShafferJason Wykoff[1,2,3,4], Kenza ArabaHailey Dodson[1,2,3,4], Camille Ehre[1,2,3,4]

[1]University of North Carolina at Chapel Hill, [2]Marsico Lung Institute, [3]Department of Pediatrics, [4]Department of Microbiology and Immunology

Cystic fibrosis (CF) is a multiorgan disease that affects mucus-producing organs expressing CFTR. Gel-forming mucins confer the viscoelastic properties of mucus and are central to CF pathogenesis. In the lungs, abnormal MUC5B and MUC5AC properties drive airway obstruction, but the link between CFTR dysfunction and mucus abnormalities remains unclear. CFTR mutations reduce Cl- and HCO3- secretion, leading to ionic imbalance, volume depletion, and mucus hyperconcentration. Reduced HCO3- secretion further compromises mucin expansion. Using highly effective modulator therapies, we identified airway hydration as the dominant biochemical factor affecting mucus properties. Additionally, RSV infection worsens CF mucus pathology due to impaired fluid secretion and increased mucus adhesion, limiting viral clearance. In the gut, CFTR dysfunction initiates mucus accumulation and causes metabolic disturbances, contributing to obstruction and dysbiosis.

Primary human bronchial epithelial cells (HBECs) from CF patients were treated with Ivacaftor or Elexacaftor-Tezacaftor-Ivacaftor (Trikafta). CFTR function was confirmed by Ussing chamber assays. Mucin biochemistry was analyzed via agarose western blotting and SEC-MALLS. Airway surface liquid (ASL) pH and mucus biophysical properties were assessed using microprobes, particle tracking microrheology (PTM), and scanning electron microscopy (SEM). RSV infection studies examined ASL height, mucus adherence, and viral mobility. Intestinal mucus properties were evaluated in CF mice, assessing Muc2 expression, bulk RNA-seq, and histological analysis.

CFTR modulators restored function, reducing total mucin concentration and relaxing the mucus network. In F508del HBECs, Trikafta treatment improved mucin clearance and rheology, with 60% of mucus remaining adherent in untreated cells versus ~25% in treated cells. These effects occurred independently of ASL pH changes. Extended hydration normalized mucus properties in untreated CF cells, highlighting hydration as a key factor in mucus regulation.

CFTR dysfunction and CF mucus abnormalities compromise RSV viral clearance. In contrast with healthy cultures, CF airway cultures infected with RSV failed to increase ASL height post-infection, suggesting CFTR-dependent fluid influx is crucial for viral clearance. RSV-infected CF cells exhibited dense, adherent mucus, limiting viral mobility. Modulator and hydration therapies reduced mucus % solids and increased mucociliary transport, enhancing viral clearance. In healthy cells, CFTR modulation also increased viral mobility, reinforcing the role of CFTR in mucosal defense.

CFTR dysfunction alters gut mucin distribution, contributing to obstruction and dysbiosis. In CF mice, Muc2 mRNA expression remained unchanged, but intestinal mucus was denser, particularly in the ileum. Bulk RNA-seq analysis showed increased tethered mucins and glycosyltransferases, suggesting altered epithelial glycocalyx composition. CF ileal tissue exhibited hypoxia, inflammation, and impaired oxidative phosphorylation and fatty acid metabolism. Paneth cell dysfunction and bacterial colonization at mucus-adherent crypts were identified as key contributors to dysbiosis and obstruction. CF mucus abnormalities stem from hydration deficits rather than pH imbalances. CFTR modulators improve mucus clearance by reducing mucin concentration and enhancing transport. RSV infection further exacerbates CF mucus pathology, with CFTR modulation improving viral clearance. In the gut, mucin adhesion and altered epithelial metabolism contribute to obstruction and inflammation. These findings underscore the importance of mucus hydration in CF pathology and therapeutic strategies.

S2.2 Changing inflammatory profiles post Trikafta

Mullen E, Murphy M, Casey M, Gunaratnam C, McElvaney NG

Irish Centre for Genetic Lung Disease, Royal College of Surgeons in Ireland, Beaumont Hospital, Dublin, Ireland

The advent of highly effective modulator therapies (HEMT has resulted in beneficial change to many of the parameters predicting premature death in people with cystic fibrosis (PWCF. Yet, it remains unclear how much residual airway inflammation will persist in PWCF on HEMT and whether the beneficial effects will decrease over times. In this presentation I will discuss the evidence for the anti-inflammatory effects of HEMT, due either to a direct CFTR effect or downstream anti-inflammatory effects. I will present data on changes in differential cell counts, protease burden, anti-protease return, cytokine changes and mucus production in the lung as well as effects of HEMT on systemic inflammation. I will also present data on the effects of HEMT on bacterial colonization and lung microbiome, outlining the difficulties in evaluating these parameters in PWCF who now no longer expectorate sputum. I will outline the inflammatory response in those PWCF unable to avail of HEMT due to their CF genotype or financial constraints and will discuss other potential therapeutic options for these individuals. I will discuss ongoing studies to evaluate inflammation, including airway bacterial and fungal colonization in PWCF on HEMT and the difficulties posed by them.

S2.3 Impact of inflammation on airway mucus properties and clearance

Marcus A. Mall[1,2,3], Tihomir Rubil[1,2,3], Greta L Harnisch[1,2,3], Christine K. Wong[1,2,3], Anita Balázs[1,2,3]

[1]Department of Pediatric Respiratory Medicine, Immunology and Critical Care Medicine, Charité - Universitätsmedizin Berlin, Berlin, Germany, [2]German Center for Lung Research (DZL), associated partner site Berlin, Berlin, Germany, [3]German Center for Child and Adolescent Health (DZKJ), partner site Berlin, Berlin, Germany

Neutrophilic inflammation, abnormal viscoelastic properties of airway mucus and impaired mucociliary clearance are key features of CF lung disease. However, the link between airway inflammation and abnormal mucus properties and clearance in CF is not well understood and it has been challenging to study the impact of inflammatory stimuli on the viscoelastic properties of the thin native mucus layer (~1 -- 20 µm) and mucociliary transport on airway surfaces.

We therefore established imaging tools for quantitative measurements of viscoelastic properties and transport velocity of the native mucus layer on the airway epithelial surface using fluorescence recovery after photobleaching (FRAP) and magnetic micro-wire rheology (MMWR), and mucociliary transport (MCT) using particle tracking. We used these techniques to study the effects of interleukin-1β (IL-1β), a key pro-inflammatory cytokine, on mucus properties and transport on highly differentiated nasal epithelial cultures from healthy individuals and patients with CF. In addition, we determined effects of pharmacological restoration of CFTR function with elexacaftor-tezacaftor-ivacaftor (ETI) in nasal cultures from CF patients carrying the F508del mutation.

Using these assays, we found that the viscoelastic properties of the native mucus layer on CF primary nasal cultures were abnormal compared to healthy cultures, even in the absence of IL-1β stimulation. Treatment with ETI improved mucus properties and mucociliary transport on CF cultures. Stimulation with IL-1β increased expression and secretion of the mucin MUC5B leading to hyperconcentration and increased viscoelasticity of the mucus layer on nasal cultures from healthy individuals and abrogated restoration of mucociliary transport on cultures from CF patients.

Collectively, our data demonstrate that FRAP and MMWR are sensitive techniques to detect changes of the viscoelastic properties of the native mucus layer covering airway surfaces that are caused by CFTR dysfunction and inflammatory stimuli. Further, our results indicate that induction of MUC5B secretion by inflammatory stimuli such as IL-1β aggravates mucus hyperviscosity in CF and impedes restoration of mucociliary clearance by ETI treatment providing important insights into the link between airway inflammation and mucus dysfunction.

Supported by the German Research Foundation (CRC 1449 -- project 431232613) and the German Federal Ministry of Education and Research (82DZL009C1).

P19

S2.4 *Ex vivo* pig lung as a new Cystic Fibrosis model for the study of *Pseudomonas aeruginosa* biofilm infection and phage therapy application

M. Cafora[1], F. Forti[2], V. Capurro[3], F. Harrison[4], F. Briani[2], A. Pistocchi[1]

[1]University of Milan, Department of Medical Biotechnology and Translational Medicine, Milan, Italy, [2]University of Milan, Department of Biosciences, Milan, Italy, [3]IRCCS Istituto Giannina Gaslini, UOC Genetica Medica, Milan, Italy, [4]University of Warwick, School of Life Sciences, Coventry, United Kingdom

Chronic bacterial infections affect individuals whose normal immune defenses are compromised, including those with cystic fibrosis (CF). *Pseudomonas aeruginosa* (*Pa*) colonization of the lower airways in people with cystic fibrosis (pwCF) leads to intractable biofilm infections. Despite repeated antibiotic administration, episodes of acute pulmonary exacerbation occur, eventually leading to death from respiratory failure. To counteract this type of antibiotic-refractory infection, phage therapy, the use of exogenous bacteriophages (or phages), is attracting increasing interest. In previous studies, we isolated and characterized a collection of phages able to kill *Pa in vitro*. A four-phage cocktail (CK φ) was able to counteract *Pa* acute infections in different animal models, although these models cannot reproduce some of the aspects of the CF airway, in particular concerning biofilm formation. Currently, most biofilm studies primarily rely on *in vitro* assays developed on abiotic surfaces, offering limited predictive value. Therefore, there is the need to develop models that can mimic the *in vivo* lung CF environment, thus reproducing the metabolic state of the pathogens.

Through this research, we have set up an *ex vivo* pig lung (EVPL) CF model, which closely mimics the physicochemical environment of human CF airways, allowing growth of *in vivo*–like biofilm. In this model, swine bronchial tissue is incubated with an artificial sputum medium that replicates the composition of CF airway fluids, allows for emulation of chronic CF pulmonary infections. We are investigating biofilm infections caused by various strains of *Pa*, including laboratory strains and strains obtained from pwCF who have been treated with modulators. The anti-biofilm potential of various phage preparations has been evaluating alone and in combination with antibiotics. We observed that phage treatment nearly eradicated biofilm formed by PAO1 and LESB58 strains in the EVPL model, also showing a synergistic effect with antibiotics, resulting in a decrease in the CFU count of biofilm biomass. Additionally, phage treatment disrupted biofilm architecture and strongly inhibited biofilm matrix production. The effectiveness of phages against biofilm growth in the EVPL model was weaker at equivalent concentrations compared to the in vitro biofilm developed on the Calgary device, suggesting the formation of a more robust biofilm in the EVPL model. Additionally, in the EVPL model the treatment with single phage preparation (DEV, *Schitoviridae*) partially eradicated the biofilm formed by *Pa* clinical isolates derived from PwCF. Furthermore, phage treatment was able to reduce intensity and spread of infection on bronchial tissue infected with the fluorescent reporter strain PAO1-GFP. A further validation of phage treatment will be performed using wild-type or CFTR mutated human primary bronchial epithelial cells.

The present study aims to demonstrate the effectiveness of the EVPL CF model in evaluating the efficacy of phages against *Pa* biofilm, addressing a critical challenge in phage therapy. The topic holds significant importance for PwCF, given the formidable resistance and persistence of *Pa*. Moreover, the implementation of a cost-effective, easy-of-use CF model has the potential to accelerate the translation potential of phage therapy into clinical practice.

P16

S2.5 Investigating the therapeutic effects of K+ channel modulators on mucus and airway surface liquid in cystic fibrosis

O. Hamed[1,2], Z. Chen[2], D.C.H. Benton[2], V. Dua[1], G.W. Moss

[1]University College London, Department of Chemical Engineering, London, United Kingdom, [2]University College London, Department of Neuroscience, Physiology and Pharmacology, London, United Kingdom

Epithelial ion transport regulates the depth of airway surface liquid (ASL), a thin fluid layer (0.1-10 µm) lining the airway epithelium. The ASL enables vital mucociliary clearance (MCC) of mucus-trapped pathogens in the upper airways via co-ordinated beating of thousands of cilia. In cystic fibrosis (CF), loss-of-function mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) cause a loss of anion secretion into the ASL, dehydrating the ASL, thus generating sticky mucus that is difficult to clear, resulting in chronic lung infections.

In recent years, CFTR correctors and potentiators have significantly improved quality of life in CF patients. However, these do not fully restore CFTR function, while patients with specific mutations are excluded from treatment. K^+ channels have been demonstrated to play an essential role in ASL regulation by setting the electrochemical driving force for anion secretion. Hence, K^+ channel modulation presents a promising therapeutic strategy for mucus rehydration in CF.

To investigate the potential benefits of K^+ channel modulation, we cultured human bronchial epithelia from CF donors with R347P/ΔF508 and ΔF508/ΔF508 mutations, treating them for 24 hours with ETI (3 µM elexacaftor, 3 µM tezacaftor, 0.5 µM ivacaftor) or ETI with a K^+ channel modulator (KM). The K^+ channel modulator tested has previously passed Phase I and Phase II clinical trials for other conditions.

To quantify the effectiveness of therapeutic target modulation in CF airway models, we used nanosensor probes mounted on a scanning ion conductance microscope (SICM) to measure key airway epithelial characteristics associated with airway disease pathology. This included mucus rheology, cilia coordination and ASL depth. Reduced ciliary co-ordination is increasingly reported as occurring in CF, contributing to impaired MCC, along with mucus rheology. Hence, we also attempted to assess whether treatment could improve ciliary co-ordination by measuring cilia beat frequency (CBF) and assessing a) CBF variance across cultures, b) average variance between all possible pairs of CBF positions within cultures, normalised by distance between the points (twin-point-median-variance, TPMV).

We measured the ASL properties of 7 ETI treated cultures (3 R347P/ΔF508 and 4 ΔF508 homozygotes) in the SICM and then treated them with ETI+KM to be examined the next day. In all 3 cultures where CBF could be measured, there was a mutation-independent, statistically significant ($p<0.05$) reduction in CBF variance and TPMV, indicating improved co-ordination of cilia in the cultures. In all 7 cultures, the variance of the spinnability decreased after ETI+KM-treatment ($p=0.008$), an effect previously reported by us with GoSlo SR-5-6, a potent activator of large-conductance calcium-activated K^+ channels. This effect was replicated when we treated CF cultures with ETI and is in line with the theory that reduced MCC is associated with wider variance in spinnability, perhaps due to the formation of mucus 'clumps' and reflecting reduced homogeneity of CF mucus.

P20

S2.6 Utilisation of human induced pluripotent stem cells for modelling cystic fibrosis lung disease *in vitro*

M.-C. Jaboreck[1], A. Balázs[2], J. Zöllner[1], N. Cleve[1], L. Czichon[1], L. von Schledorn[1], J. Hegermann[3], J.C. Nawroth[4], D. Roth[4], M. Mielenz[5], S. Hedtfeld [5], F. Stanke[5], T. Rubil[2], F. Ius[6], D. Jonigk[7], J.W. Hanrahan[8], R. Olmer[1], M.A. Mall[2], S. Merkert[1], U. Martin[1]

[1]Hannover Medical School (MHH), Leibniz Research Laboratories for Biotechnology and Artificial Organs (LEBAO), Hannover, Germany, [2]Charité - Universitätsmedizin, Department of Pediatric Respiratory Medicine, Immunology and Critical Care Medicine, Berlin, Germany, [3]Hannover Medical School (MHH), Core facility for electron microscopy, Hannover, Germany, [4]Helmholtz Munich, Helmholtz Pioneer Campus and Institute of Biological and Medical Imaging, München, Germany, [5]Hannover Medical School (MHH), Clinic for Pediatric Pneumology, Allergology and Neonatology, Hannover, Germany, [6]Hannover Medical School (MHH), Transplantationszentrum, Clinic for Cardiothoracic-, Transplantation and Vasuclar Surgery, Hannover, Germany, [7]Universitätsklinikum Aachen, Reference Centre for Lung Pathology, Aachen, Germany, [8]McGill University, Department of Physiology, Montreal, Canada

Cystic Fibrosis (CF) is characterised by a complex multi-organ pathophysiology and lung disease as the major cause of mortality. Despite improved treatment options, the development of alternative drugs and individualised therapies is still urgently needed to address phenotypic variations and untreatable CFTR mutations. Intestinal organoids have been used as a CF model and personalised drug screening system but reflect CF lung disease only to a limited extent. Primary airway cells are useful, but only available in small quantities.
In contrast, patient-specific human induced pluripotent stem cells (hiPSCs), with their unlimited potential for proliferation and differentiation, and the possibility to use gene corrected clonal lines equipped with useful reporter transgenes, allow for the generation of organotypic culture systems comprising all relevant airway cell types. However, it has remained unclear to what extent such air-liquid-interface (ALI) cultures derived from hiPSCs recapitulate the pathophysiology of CF lung disease.
We now provide a comprehensive characterisation of hiPSC- derived airway (iALI) cultures and demonstrate the utility of iALI culture for CF research. Our data show a high similarity between iALI cultures and primary airway (pALI) cultures, the current gold standard model system. We compared mRNA and protein expression, mucus (ultra)structure and height, ion channel function and ciliary beat frequency to characterise the manifestation of the CF disease phenotype in iALI cultures and to test disease recovery by CFTR modulator treatment. The application of an automatable ciliary beat frequency assay, which is largely unaffected by common variable cellular impurities, facilitates sensitive measurement of mucociliary function as the major pathomechanism of CF lung disease. iALI cultures thus represent a valuable system for modelling CF lung disease and for personalised drug development with an unlimited supply of patient-specific cells.

27 March — 16:15–18:00

Symposium 03 - CFTR and cancer / ageing

S3.1 Cystic fibrosis associated colorectal cancer; a complex and urgent challenge to tackle

S. Spelier[1,2], M. Bani Baher[1,2], H. Sonneveld[1,2], R. Hofland[3], J. M. Beekman[1,2]

[1]Department of Pediatric Respiratory Medicine, Wilhelmina Children's Hospital, University Medical Center, Utrecht University, 3584, EA, Utrecht, The Netherlands, [2]Regenerative Medicine Utrecht, University Medical Center, Utrecht University, 3584, CT, Utrecht, The Netherlands, [3]Department of Respiratory Medicine, University Medical Center, Cystic Fibrosis Center, Utrecht University, 3584 EA Utrecht, The Netherlands

The last decade of CF research is characterized by a massive paradigm shift in terms of the development of highly effective modulator treatments (HEMTs), that effectively treat various CFTR mutations. Whilst this is clearly major progress, the aging CF population faces new difficulties. Recent studies show that a third of all adult CF patients suffer from a range of comorbidities such as a large increased risk of developing several epithelial cancers, including colorectal cancer (CRC). The prevalence of CRC in the non-CF population between 40-49 years old is 2-5%, whereas in CF patients the CRC prevalence is around 23%. Understanding how dysregulated CFTR could result in the increased chance of developing CRC is pivotal in order to prevent the build-up of polyps, potential outgrowth of polyps and as such advancing care and quality of life for pwCF.

In line with this increased prevalence of CRC in pwCF, various animal and cell line models point out the connection between CFTR-loss of function (LoF) and dysregulation of intestinal stem cells. However, results are contradictory due to the complexity of both CFTR-LoF as well as CRC development. Consequences of CFTR-LoF can be linked to cell-autonomous effects as well as indirect effects, such as the pro-inflammatory state of the gastro-intestinal system.

In our study, we focus on thorough characterization of cell-autonomous effects of CFTR-LoF by exploiting intestinal organoid (PDIO) cultures isolated from rectal biopsies. We created CRISPR-engineered organoid isogenic organoid lines to compare wildtype CFTR with CFTR-LoF within the same genetic background. This resource offers a unique opportunity to characterize cell-autonomous consequences of CFTR-LoF. First pilot experiments indeed phenotypical differences associated with alterations in cellular differentiation, and characterization of proliferation is currently ongoing. Furthermore bulk RNA sequencing of CFTR-WT and CFTR-KO cells underlines interesting leads, such as the decreased expression of WNT pathway inhibitor DKK1 upon CFTR-LoF, which could associate with enhanced proliferative capacities. We are furthermore currently generating a biobank of polyp-material of pwCF and non-CF controls, which will allow for a deeper understanding of polyp and CRC characteristics in the context of CF.

Overall, we believe in this new era of CF-research it is pivotal to understand emerging topics such as the increased risk of CRC, which will ultimately aid in improving the quality of life for pwCF.

S3.2 Growing older with CF: new challenges for CF care and research

Isabelle Fajac

Cochin Hospital, Physiology Department, Paris, France

No abstract submitted.

S3.3 Cardiovascular disease and CF; Should we be concerned?

Damian Downey

Wellcome-Wolfson Institute for Experimental Medicine, Queen's University Belfast, UK

Introduction: The impact of CFTR modulators has dramatically improved the lives of people with CF (pwCF) with Elexacaftor/Tezacaftor/Ivacaftor (ETI) potentially allowing pwCF to achieve a near-normal life expectancy. However, one of the emerging challenges faced by an ageing CF population is the increased risk of cardiovascular disease (CVD). The most common CVD conditions observed in pwCF include atherosclerosis, hypertension and heart failure; however, other clinical sequalae include effects on the aorta, pulmonary hypertension, and peripheral vascular disease. Therefore, with an ageing CF population, increasing weight, CF diabetes (CFD), and dyslipidaemia, there is a significant concern of a cardiovascular time bomb in CF.

CF and CVD: PwCF have a higher risk of major adverse cardiac events than the matched general population and a higher relative risk compared with other inflammatory conditions considered "high risk" for CVD. There are also well-documented cases of CVD and myocardial infarction in CF and with an ageing CF population the absolute risk is anticipated to escalate. Using QRISK3, a widely used tool as part of CVD primary prevention in the UK, it was demonstrated that the heart age of pwCF was 8 years more than their actual age, suggesting an increased risk of CVD. Young pwCF already show increased arterial stiffness and signs of diminished left and right ventricular dysfunction. A lower left ventricular ejection is associated with worse outcomes in CF.

There is also clinical evidence of vascular endothelial cell (EC) dysfunction in young pwCF. This is important as it is an early atherogenic event and a risk factor for CVD. Inflammation is a promoter of atherosclerosis, and pwCF have both organ specific and systemic inflammation. CRP has been shown in pwCF to correlate with disease activity. Its elevation is associated with atherosclerosis, angina, myocardial infarction, and recurrent CV events in non-CF cohorts. Additionally, approximately 30% of adults with CF diabetes, and insulin resistance has been shown to be associated with CVD and events in non-CF individuals. There are reports of myocardial infarction in young women with CF and CFD.

CFTR modulators and CVD: Following the introduction of ETI, a retrospective observational review found the mean rate of change in body weight was 4.43 kg per year, with significant increases in both systolic and diastolic blood pressure. A case series has documented the onset of systemic arterial hypertension in several pwCF within the first few weeks of starting ETI, all requiring cardiac evaluation and commencement of chronic anti-hypertensive therapy. Dyslipidaemia also occurs in pwCF, with cholesterol and triglycerides increasing with advancing age and BMI. Those on ETI also have elevated triglyceride cholesterol ratios, suggesting an increased risk of CVD.

Conclusion: As pwCF age, it is expected that non-pulmonary complications will become increasingly prevalent and an increasing part of care delivery. There is concern of progressive weight gain, hypertension, inflammation, dyslipidaemia and CFD in a population growing older on ETI. CVD is underexplored in CF and should be a research focus for the coming years.

P25

S3.4 Elexacaftor/tezacaftor/ivacaftor CFTR modulators mitigate senescence in cystic fibrosis

A.M. Hristodor[1], T. Gunawardena[2], M. Borgatti[3], A. Vella[4], C. Boni[1], D. Olioso[5], F. Quiri[1], G. Lippi[5], T. Moraes[6,7], M. Cipolli[1], V. Bezzerri[1,8]

[1]Azienda Ospedaliera Universitaria Integrata di Verona, Cystic Fibrosis Center, Verona, Italy, [2]Hospital for Sick Children, Department of Molecular Medicine, Research Institute, Toronto, Canada, [3]University of Ferrara, Department of Life Sciences and Biotechnology, Ferrara, Canada, [4]Azienda Ospedaliera Universitaria Integrata di Verona, Unit of Immunology, Verona, Canada, [5]University of Verona, Section of Clinical Biochemistry, Department of Engineering for Innovation Medicine, Verona, Italy, [6]Hospital for Sick Children, Division of Respiratory Medicine, Department of Paediatrics, Toronto, Canada, [7]Hospital for Sick Children, Department of Translational Medicine, Research Institute, Toronto, Canada, [8]Link Campus University, Department of Life Sciences, Health, and Health Professions, Rome, Italy

Impaired CFTR function causes loss of chloride and bicarbonate efflux across epithelia, determining airway surface liquid dehydration, oxidative stress, and mucus accumulation into the bronchial lumen. This microenvironment promotes subsequent bacterial infections, mainly sustained by *P. aeruginosa*, that amplify the inflammation.

Senescence has been initially described as a state of irreversible cell cycle arrest induced as a response to oxidative stress, DNA damage, or physiologic aging. Senescence may act as a double-edged sword, showing both beneficial and detrimental effects. In the short term, senescence acts as a protective mechanism, supporting wound healing and preventing damaged cells from proliferating. On the other hand, the chronic accumulation of senescent cells releasing proinflammatory factors, collectively referred to as the Senescence-Associated Secretory Phenotype (SASP), further contribute to low-grade chronic inflammation. This leads to the "inflammaging" process, increasing the risk of age-related disorders, fibrosis, cancer, and autoimmune diseases. Thus, we sought to clarify if senescence plays a relevant role in CF pathophysiology, representing a main driving-force of the constitutional inflammatory vicious cycle. Then, we evaluated the effect of elexacaftor/tezacaftor/ivacaftor (ETI) on the expression of several senescence markers in various CF airway epithelia.

Normal and CF bronchial epithelial cell lines, as well as primary air-liquid interface (ALI)-differentiated human bronchial (hBECs) and nasal (hNECs) epithelial cells, homozygous for the F508del CFTR variant, with similar donor ages and culture passages, were used. Immunofluorescence, cell stress proteome profiling array (R&D, Minneapolis) and western blot were used to evaluate senescence biomarkers in protein extracts from hBECs and hNECs. We found elevated levels of tumor suppressor p53 and its downstream effectors p21 and p16 in CF bronchial epithelia. Interestingly, the inhibition of CFTR-dependent chloride efflux sustained by CFTR(inh)-172 further increased p53 and p16 levels in healthy hBECs. Several stress-related proteins linked to senescence, such as COX-2, Bcl-2, HSP27, HSP60, p-JNK, and NF-κB, were elevated in CF hBECs. Higher levels of SASP-related cytokines and chemokines (IL-8, IL-1Rα, IP-10, GM-CSF) were also noted. Morphologically, CF bronchial epithelial cells were enlarged and flattened, showing increased expression of cytoskeletal component vimentin and decreased Lamin B1 compared to non-CF cells, according to the senescence hypothesis.

Most importantly, while CFTRinh-172 induced vimentin expression in healthy hNECs, ETI restored normal levels of vimentin and Lamin B1 in CF hNECs by modulating p53 pathway.

Of note, SASP soluble mediators were analyzed in plasma samples from 50 patients with CF (homozygous for F508del mutation) before and after 12 months of ETI therapy. Plasmatic levels of several SASP mediators (IL-1β, IL-6, IL-8, G-CSF, MIP-1α, TNFα, and IL-17) were significantly reduced upon ETI therapy.

These results highlight that: i) senescence biomarkers are constitutively elevated across various CF airway models: ii) senescence may act as a major driver of the constitutive inflammation observed in CF: iii) ETI can partially mitigate senescence biomarkers both in vitro and in vivo, highlighting potential benefits that may go far beyond the expected ones.

P22

S3.5 Pancreatic cancer growth rate may be susceptible to variations in CFTR expression

R. Ibáñez[1], J.P. Muñoz[1], M. Corbacho[2], J. Barquinero[3], M.T. Salcedo[4], L. Campderros[1], E.C. Vaquero[2], X. Molero[1]

[1]Vall d'Hebron Institute of Reserach (VHIR), Pathophysiology and treatment of pancreatic disorders, Barcelona, Spain, [2]ICMDM, Hospital Clínic, IDIBAPS, Gastroenterology Department, Barcelona, Spain, [3]Vall d'Hebron Institute of Reserach (VHIR), Gene and Cell Therapy Department, Barcelona, Spain, [4]Hospital Universitari Vall d'Hebron, Pathology Department, Barcelona, Spain

Background: Cystic Fibrosis patients are at higher risk of developing cancer of the gastrointestinal tract that shows accelerated growth. Pancreatic stellate cells (PSCs) are active members of the cancer microenvironment. Moreover, subcutaneous injection of pancreatic cancer cells along with PSCs results in increased tumour growth. We have shown CFTR is expressed and regulated in PSCs, and reduced CFTR expression is linked to the acquisition of a proliferative and proinflammatory phenotype. Reduced expression of CFTR may contribute to generation of more aggressive pancreatic tumours.

Methods: CFTR expression was examined in human pancreatic cancer cell lines MiaPaca2, Panc-1, Capan2 and BxPC3, and in murine pancreatic cancer cells KPC (K-ras and p53 mutated) by means of ddPCR. Cancer cell lines were ranked from more aggressive to less aggressive according to their doubling proliferation time rate and differentiation grade (E-cadherin/Zeb-1 expression). PSCs were isolated from wild-type (wt) or CFTR-KO mice and their proliferation rate examined. The proliferation of luciferase-expressing KPC cells was examined in stand alone cultures, or after being co-cultured with either wt-PSC or CFTR-KO PSC. Allogeneic subcutaneous pancreatic tumours were induced in normal mice by injecting a mixture of KPC and PSCs (either wild-type or CFTR-KO) in Matrigel /DMEM media. Tumour growth was monitored for 28 days. Before sacrifice, mice were submitted for computerized tomography examination to extract radiomic features. Tumours were then excised, weighted and processed for histopathology.

Results: Despite being of "ductal" origin, human pancreatic ductal adenocarcinoma cell lines expressed very low levels of CFTR. CFTR expression was inversely associated with the degree of differentiation, being undetected in MiaPaca2 cells, barely expressed in Panc-1/Capan2 cells, and better detected in BxPC3 cells. The well-differentiated murine pancreatic cancer cell line KPC showed the largest CFTR expression. PSCs devoid of CFTR (CFTR-KO PSCs) showed higher rates of proliferation than wild-type PSCs. In addition, pancreatic cancer cells KPC showed higher proliferation rates when co-cultured with CFTR-KO PSCs, as compared to KPCs co-cultured with wild-type PSCs or to KPC cultured alone. Pancreatic tumours generated using CFTR-KO PSCs had larger volumetric growth rate and final weight than tumours developed using wild-type PSCs. Pathology examination disclosed greater disorganized structure in tumours from CFTR-KO PSCs that showed enhanced radiomic features associated with tumour aggressiveness (lesser entropy, contrast and cluster tendency, and higher energy and nonuniformity), which supported the pathology findings. In summary, reduced CFTR expression matches cell dedifferentiation and proloiferation rates in pancreatic cancer cell lines, and PSCs defective in CFTR enhance pancreatic cancer growth, both in vitro and in vivo.

Conclusion: CFTR expression levels impacts on pancreatic cancer growth rate and on the tumour inner structure. Global CFTR activity may be a relevant factor modulating cancer aggressiveness.

P21

S3.6 Development of new models to study the impact of CFTR dysfunction on osteocytes in cystic fibrosis-related bone disease

L. Thoraval [1], C. Guillaume[1], L. Hamon[1], G. Bouet-Chalon[2], A. Vanden-Bossche[3], D. Marchat[2], F. Velard[1]

[1]Université de Reims Champagne-Ardenne, UR BIOS, Reims, France, [2]Mines Saint-Etienne, Univ Jean Monnet, Etablissement Français du Sang, INSERM, U 1059 Sainbiose, Saint-Etienne, France, [3]Université Jean Monnet Saint-Étienne, INSERM, Mines Saint Etienne, U 1059 Sainbiose, Saint-Etienne, France

Objectives: Cystic fibrosis-related bone disease (CFBD) occurs in 20-50% of adults with cystic fibrosis (CF). CFBD patients present with low bone density and an increased risk of fractures, affecting their quality of life. Understanding the impact of CFTR defect on bone physiology is essential to propose more effective therapeutic strategies. Previous studies have already shown CFTR dysfunction effect on the activity of osteoblasts [Velard, 2014; 2015; Delion, 2016; Dumortier, 2025] and osteoclasts [Jourdain, 2021], leaving osteocytes out in the cold. Far from being just a passive placeholder in bone, osteocytes are long-lived cells within the bone matrix that perform a variety of functions, including the control of bone remodeling [Dallas, 2013]. Their abnormal behavior has been described as contributing to bone tissue deterioration in post-menopausal osteoporosis and skeletal aging [Jilka, 2016]. The aim of this work is, i) to develop an innovative *in vitro* model to study the impact of CFTR dysfunction on osteocytes in CF, ii) to analyze osteocytes *ex vivo* in CF(F508delCFTR) and non-CF mice, in an ovariectomy (OVX)-induced osteoporosis model.

Methods: Human primary mesenchymal stem cells (MSC) (n=3 independent donors) are cultured on textured bioceramics for 28 days [Jugnet, 2017], with or without CFTR pharmacological inhibitors (inh-172, BPO-27). Expression of osteocyte genes (*BGLAP*, *SOST*, *DMP1*, *FGF23*) is assessed by RT-qPCR and the associated protein secretion by ELISA. Stainings (Von Kossa, Hematoxylin Eosin, Masson's Trichrome) and fluorescent labeling (phalloidin-AlexaFluor®488, DAPI) are performed to identify mineralized and organic matrix production as well *in situ* cells morphology. Eight-week-old CF (n=7) or non-CF (n=6) mice undergo surgical ovariectomy (OVX) to mimic post-menopausal osteoporosis. Tibiae from control or OVX mice are collected, fixed in ethanol, decalcified and prepared for cryotomy and enhanced-resolution confocal analysis, or optical transparency for analysis by light-sheet microscopy.

Results: The first *in vitro* differentiation tests and *ex vivo* analyses are carried out to enable us to optimize the methods. Osteogenic medium as well as seeding conditions are optimized to obtain cells expressing osteocyte-markers at mRNA level. Impact of CFTR inhibitors on this osteocyte-like phenotype is currently under investigation. Analysis of fluorescent labeling samples with phalloidin-DAPI has demonstrated our ability to image *ex vivo* osteocytes and their cytoplasmic extensions using confocal microscopy. We successfully perform OVX surgery (n=7 CF and n=6 non-CF mice), evidencing blunted bone microarchitecture in CF condition worsen by OVX. In addition, staining of *ex vivo* samples and their analysis by microscopy will reinforce our understanding of osteocyte phenotype and osteocyte lacuna-canalicular network structure in CF *vs* non-CF conditions.

Conclusion: Future work using iPSC-derived MSC will allow us to gain insights on native CFTR mutation effect in comparison with functional inhibition of the channel to shed light on the involvement of osteocytes in the deregulation of bone homeostasis in CF patients, reinforcing this work evidence that CFTR loss of function in CF induced global dysregulation of all bone cells.

28 March — 08:45–10:30

Symposium 04 - CFTR structure and function and therapeutic relevance

S4.1 Elexacaftor-tezacaftor-ivacaftor rescue of F508del-CFTR chloride channels

David N. Sheppard

School of Physiology, Pharmacology and Neuroscience, University of Bristol, Bristol, UK

Elexacaftor-tezacaftor-ivacaftor has transformed the treatment of people with cystic fibrosis (CF) and the predominant cystic fibrosis transmembrane conductance regulator (CFTR) variant F508del by delivering the faulty channel to the plasma membrane and potentiating its activity. However, the action of elexacaftor-tezacaftor-ivacaftor on the single-channel behaviour of F508del-CFTR is unknown. In this presentation, the impact of elexacaftor-tezacaftor-ivacaftor on individual and populations of human F508del-CFTR Cl^- channels, studied with electrophysiological techniques will be discussed. Chronic treatment of cells heterologously expressing F508del-CFTR with elexacaftor-tezacaftor-ivacaftor stabilised CFTR-mediated Cl^- currents in the apical membrane of epithelia and individual Cl^- channels in cell-free membrane patches. Elexacaftor-tezacaftor-ivacaftor, but not pairs of CFTR modulators, prevented the loss of current flow through open F508del-CFTR Cl^- channels in cell-free membrane patches. However, neither elexacaftor-tezacaftor-ivacaftor nor pairs of CFTR modulators conferred the gating behaviour of wild-type on F508del-CFTR. In conclusion, chronic treatment of F508del-CFTR with elexacaftor-tezacaftor-ivacaftor stabilises its open-channel conformation without restoring channel activity to wild-type levels. Thus, F508del-CFTR rescued by elexacaftor-tezacaftor-ivacaftor retains structural defects, which require new combination therapies for complete correction.

S4.2 Understanding and fostering CFTR potentiation

Ahmad Elbahnsi[1,2], Solène Castanier[3], Alexandre Hinzpeter[3], Jean-Paul Mornon[1], Isabelle Callebaut[1]

[1]Sorbonne Université, Muséum National d'Histoire Naturelle, CNRS UMR 7590, IMPMC, Paris, France , [2]Université Paris Cité, CNRS UMR 8038 CiTCoM, Inserm U1268 MCTR, Paris, France, [3]Université Paris Cité, INSERM U1151, CNRS UMR 8253, Institut Necker Enfants Malades, Paris, France

The CFTR protein has evolved from the mold of an ABC exporter, acquiring specific features related to its function as an anion channel (1). These include (i) a degenerate intracellular gate, with the appearance of at least one lateral portal, which allows the flow of ions from the cytoplasm, (ii) an asymmetry within transmembrane (TM) domains, including an unwound segment at the level of helix TM8, (iii) pore-lining and pore-stabilizing residues, and (iv) a large regulatory region, whose phosphorylation by protein kinase A regulates gating. Despite knowledge of the three-dimensional structure of the CFTR protein in its phosphorylated, ATP-bound form, in the presence of the VX-770 potentiator and the inh-172 inhibitor (2), the mechanisms underlying channel activity remain incompletely understood at the molecular level. However, this knowledge is essential for assessing the impact of mutations and how mutation-induced defects can be corrected by the use of modulators.

A better understanding of these mechanisms can be achieved by coupling the identification of potentiator binding sites with that of amino acids that affect channel sensitivity to potentiation or activate the channel (gain-of-function).Combining these data with molecular modelling and molecular dynamics simulations has the potential to highlight critical regions within TM domains, including extracellular loops, and to reveal key allosteric pathways intricately involved in potentiation mechanisms (3).

1. Infield et al. J Gen Physiol 2021; Hwang et al. J Cyst Fibr 2022, Farinha & Callebaut Biosci Rep 2022.

2. Liu et al. Science 2017; Young et al. Proc Natl Acad Sci USA 2024; Gao et al. Nat Comm 2024.

3. Castanier, Elbahnsi et al. Cell. Mol Life Sci 2024; Castanier, Elbahnsi et al. submitted, Elbahnsi et al. in preparation.

S4.3 Structure/function mechanisms of CFTR modulators

Tzyh-Chang Hwang[1,2]

[1]Institute of Pharmacology, National Yang Ming Chiao Tung University, Taiwan., [2]Dalton Cardiovascular Research Center, Department of Medical Pharmacology and Physiology, University of Missouri-Columbia, USA

Opening and closing (i.e., gating) of the CFTR chloride channel can be modulated by small molecules that bind directly to the CFTR protein. These modulators work by either increasing or decreasing the activity of CFTR---hence named CFTR potentiators and inhibitors respectively. While CFTR potentiators such as ivacaftor (VX-770) play a major role in the treatment of cystic fibrosis, CFTR inhibitors (e.g., CFTRinh-172) bear the potential for the treatment of secretory diarrhea. In either case, to become an effective drug candidate, the compound must possess high potency (i.e., low IC50 or EC50) as well as high efficacy (large maximum effect) so that an optimal clinical benefit can be attained at a lowest concentration without eliciting side effects. Our previous studies (Yeh et al., 2017) suggested that CFTR potentiators bind more tightly to the open channel than to the closed channel. This state-dependent binding scheme indicates that the potency of the compound depends on the binding affinities to both the open and closed states; whereas the efficacy is decided by the difference in affinity between these two states for the compound. In the current presentation, I will use available cryo-EM structures of CFTR/potentiator complexes to illustrate what we have learned at a molecular level about the interactions between these compounds and CFTR, and how an integration of the structure/function mechanism may help develop more potent and efficacious CFTR potentiators. For CFTR inhibitors, our previous report (Kopeikin et al., 2010) suggested that CFTRinh-172 works mainly by inducing a conformational change that shuts the chloride conducting pathway in CFTR. Recent cryo-EM data (Young et al., 2024; Gao et al., 2024) indeed provide structural evidence for such conformational changes in CFTR's transmembrane domains. I will present our most recent electrophysiological data to show how CFTR inhibitors inhibit CFTR function through a two-step process. These single-channel data also lead us to conclude that both the potency and efficacy of CFTR-inh-172 depends mainly on the second step or the conformational changes induced by inhibitor binding. Through a thorough understanding of the structure/function mechanisms of CFTR modulators, we are at the vantage point to materialize structure-based drug design for the treatment of CFTR-related diseases.

P54

S4.4 A novel active conformation of CFTR may drive functional recovery of F508del-mutant

C. Govaerts[1], M. Overtus[1], T. Rubil[2], B. Loughlin[3], Z. Rich[3], M. Rodrat[4,5], Z. Yang[6], A. Balazs[2], J.C. Kappes[6], M. Mall[2], D.N. Sheppard[4], J.F. Hunt[3]

[1]Université Libre de Bruxelles, Biochemistry and Structural Biology, Brussels, Belgium, [2]Charité-Universitätmedizin Berlin, Pneumologie, Immunologie and Intensivmedizin, Berlin, Germany, [3]Columbia University, Department of Biological Sciences, New York, United States, [4]University of Bristol, Pharmacology and Neurosciences, Bristol, United Kingdom, [5]Mahidol University, Institute of Molecular Biosciences, Nakhon Pathom, Thailand, [6]University of Alabama School of Medicine, Heersink School of Medicine, Birmingham, United States

The F508del mutation in Cystic Fibrosis Transmembrane Conductance Regulator (CFTR), the leading cause of Cystic Fibrosis (CF), disrupts protein folding and stability, severely impairing chloride channel function. Despite advancements in treatment with the modulators elexacaftor, tezacaftor, and ivacaftor (ETI), current therapies fail to fully restore CFTR activity. In this study, we explored the structural and functional mechanisms underlying CFTR activity by combining ETI with the CFTR-stabilizing nanobody T2a, delivered via lipid nanoparticles (LNPs). Co-expression of T2a, which thermally stabilizes the nucleotide-binding domain 1 (NBD1), enhances mutant CFTR expression, maturation, and activity.

Functional assays demonstrated that the combination of T2a and ETI not only restores mutant CFTR activity but also stabilizes channel function over prolonged periods, overcoming the thermal instability that limits ETI efficacy. Cryo-electron microscopy revealed two distinct T2a-bound CFTR conformations: an inactive "V-shaped" state, where T2a prevents canonical NBD1-NBD2 dimerization, and a novel active state characterized by NBD1 detachment.

This latter conformation reorganizes the transmembrane helices into a dynamic, pore-like structure compatible with ion conduction. Notably, binding of T2a promotes the emergence of subconductance states, rarely observed in wild-type CFTR but prominent in F508del-CFTR. These states, which represent partially open channel configurations, are likely intermediates facilitated by NBD1 detachment that enable dynamic reorganization of the CFTR transmembrane region.

These findings redefine our understanding of CFTR gating and channel function, particularly for mutant CFTR, and underscore the therapeutic potential of stabilizing nanobodies to enhance both structural integrity and functional output in protein trafficking diseases.

P30

S4.5 In silico, in vitro and ex vivo characterization of CFTR pathogenic variants localized in the Fourth Intracellular Loop and their rescue by modulators

E. Pesce [1], V. Tomati[1], V. Capurro[1], M. Lena[2], C. Pastorino[1], M. Astore[3], S. Kuyyucak[3], B. Chevalier[4,5], E. Sondo[1], F. Cresta[6], V. Terlizzi[7], S. Costa[8], M.C. Lucanto[8], V. Daccò[9], L. Claut[9], F. Ficili[10], R. Bocciardi[1,2], F. Zara[1,2], C. Castellani[6], L.J. Galietta[11], S.A. Waters[12,13,14], A. Hinzpeter[4,5], N. Pedemonte[1]

[1]IRCCS Istituto Giannina Gaslini, UOC Genetica Medica, Genova, Italy, [2]University of Genoa, Department of Neurosciences, Rehabilitation, Ophthalmology, Genetics, Maternal and Child Health (DINOGMI), Genova, Italy, [3]School of Physics, University of Sydney, Sydney, Australia, [4]Institut Necker Enfants Malades, INSERM, CNRS, Paris, France, [5]Université Paris-Cité, Paris, France, [6] IRCCS Istituto Giannina Gaslini, UOSD Centro Fibrosi Cistica, Genova, Italy , [7]Meyer Children's Hospital IRCCS, Cystic Fibrosis Regional Reference Center, Department of Pediatric Medicine, Firenze, Italy, [8]Azienda Ospedaliera Universitaria Policlinico G. Martino, Centro Hub Fibrosi Cistica, Messina, Italy 8, [9]Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico, Department of Pediatrics, Cystic Fibrosis Center, Milano, Italy 9, [10]Ospedale Giovanni Di Cristina, Cystic Fibrosis Center, Palermo, Italy, [11]Telethon Institute of Genetics and Medicine (TIGEM), Pozzuoli, Italy, [12]Faculty of Medicine and Health, School of Clinical Medicine, Discipline of Paediatrics and Child Health, Sydney, Australia, [13]Molecular and Integrative Cystic Fibrosis Research Centre, Sydney, Australia, [14] Sydney Children's Hospital, Department of Respiratory Medicine, Sydney, Australia

Cystic fibrosis (CF) is due to loss-of-function variants of the CFTR chloride/bicarbonate channel. The most effective treatment for people with CF (pwCF) carrying the F508del mutation (accounting for 70% of the pathogenetic alleles) is the triple combination of Elexacaftor-Tezacaftor-Ivacaftor (ETI). Although initially developed for F508del, CFTR modulators can correct the underlying defect(s) in other CFTR mutants. While in the USA, ETI has been approved for 271 mutations other than F508del, in Europe, its approval is limited to F508del. The use of disease-relevant predictive models such as patient-derived human nasal epithelial cells (HNEC) allow to investigate the response to CFTR modulators of specific genotypes, possibly supporting patients' access to treatment.

Previous studies have investigated the impact of several missense variants within the Intracellular Loop 4 (ICL4) on CFTR protein maturation and channel activity. While some of these variants, such as R1066H or L1077P, are already deemed eligible for ETI therapy, others, such as L1065P and R1066C, remain poorly investigated. Given their frequency in Italy and other Southern European countries, we aimed to collect evidence to characterize their functional defects and demonstrate their rescue by CFTR modulators in disease-relevant cell models. To this aim, we performed a detailed analysis of selected variants in the ICL4 using computational, functional and biochemical methodologies, to understand their impact on CFTR structure and function. Mutations affecting L1065, R1066, and L1077 result in disruptions of hydrophobic interactions, salt bridges, and helical integrity, respectively, leading to compromised structural stability of CFTR. Among the mutations analyzed, L1065P appears to induce the most significant conformational change, characterized by increased flexibility and loss of helix formation within the hydrophobic pocket, while the disruption of the salt bridge by R1066C and the helical destabilization by L1077P further underscore the role of ICL4 in maintaining CFTR's conformational stability. Analyses of single variants expressed heterologously in immortalized bronchial cells showed that, upon ETI, rescued activity for both L1065P and R1066C was close to 50% of the wild-type CFTR activity. Biochemical studies of ICL4 variants expression pattern in CFBE41o- cells, following treatment for 24 h, demonstrate the appearance of the band C, corresponding to mature, fully glycosylated protein, with no changes in the immature band. Cell surface measurements of CFTR performed using the HiBiT complementation assay in transiently transfected HEK293 cells confirmed partial restoration of CFTR surface expression, in the same range as observed with F508del. Finally, our study provides evidence in primary nasal cells from a cohort of pwCF that L1065P and R1066C, can be effectively rescued by ETI. Upon treatment with modulators, the CFTR-mediated current averaged around 25%-45% of the activity measured in non-CF epithelia. Although the observed rescue for L1065P and R1066C was smaller than that of the F508del (40-65% of the normal activity), it should fall in a range predicted, by various studies, to provide a clinical benefit.

Supported by Fondazione Ricerca Fibrosi Cistica grants FFC #9/2019 and FFC #10/2021, Italian Ministry of Health grants GR-2018–12367126 and PNRR-MR1-2023-12378412 and Cystic Fibrosis Foundation grant PEDEMO20G0.

P34

S4.6 Novel class 3 CFTR correctors suitable for combinatorial treatments in cystic fibrosis

M. Barreca[1], F. Lo Mascolo[1], A. Lipani[1], S. Giuffrida[1], V. Spanò[1], M.V. Raimondi[1], A. Montalbano[1], P. Barraja[1], M. Renda[2], A. Borrelli[2], A. Venturini[2], D. Guidone[2], M. Genovese[2], L.J.V. Galietta[2], F. Bertozzi[3], T. Bandiera[3]

[1]University of Palermo, Department of Biological, Chemical and Pharmaceutical Sciences and Technologies (STEBICEF), Palermo, Italy, [2]Telethon Institute of Genetics and Medicine (TIGEM), Pozzuoli (NA), Italy, [3]Istituto Italiano di Tecnologia, Genova, Italy

Significant progresses have been achieved in the pharmacological treatment of cystic fibrosis (CF) with the development of Kaftrio, a triple combination of two correctors (VX-661, VX-445) and one potentiator (VX-770). However, the development of new CFTR modulators for the rescue of F508del and other CF mutations is an important goal to maximize mutant CFTR rescue.

We recently discovered a new family of CFTR correctors, named PP compounds, effective for the rescue of F508del-CFTR (1). We are presently running a multiparametric optimization of PP compounds through iterative cycles of chemical synthesis and functional evaluation. In parallel, we are also investigating another family of correctors, SH compounds, derived from PP compounds using a scaffold hopping approach. With respect to PP family, SH compounds feature a higher conformational flexibility. To assess potency and maximal efficacy, all new synthetized compounds were screened at multiple concentrations (0.1-10 μM) using the HS-YFP functional assay on CFBE41o- cells expressing F508del-CFTR mutation. So far, we have evaluated 450 compounds from which several effective compounds emerged with EC_{50} values at sub micromolar level. For the most active compounds, efficacy was also confirmed in primary airway epithelial cells derived from CF patients. Selected candidates were evaluated for *in vivo* pharmacokinetic profiling in rodents, for possible human use. Mechanistic studies have shown that PP and SH compounds act as "class 3 correctors", able to produce a strong synergistic/additive effect when combined with class 1 agents such as VX-809 and VX-661. We tested the efficacy and potency of two selected compounds, PP028 and SH157A, when combined with another type of class 1 correctors, characterized by single digit nanomolar potency (2). The combination of ARN22081, ARN22151, or ARN22361 with PP028 or SH157A markedly enhanced F508del-CFTR rescue confirming the possibility of synergism. Interestingly, the inclusion of PP028/SH157A compounds caused an increase in the potency of ARN correctors, with a shift of the EC_{50} values to the sub nanomolar range. These results confirm the ability of the PP and SH compounds to synergize with ARN correctors thus indicating the possibility to develop novel combination for optimal rescue of mutant CFTR.

1) Renda et al. Sci Rep 13:7604, 2023; 2) Pedemonte et al., Sci Adv 6:eaay9669, 2020.

Acknowledgement: development of PP and SH compounds is supported by a grant (Molecole 3.0) from the Italian Cystic Fibrosis Research Foundation (FFC ricerca). ARN correctors were also developed with the support of FFC ricerca.

28 March — 11:00–12:45

Symposium 05 - CFRD from different perspectives: How do pwCF, clinicians, researchers deal with diabetes in CF? A patient organisation initiated symposium

S5.1 CFRD genetics: from pathophysiology to personalized medicine

Blackman, Scott M.

Johns Hopkins University School of Medicine, Baltimore, MD USA

CF-related diabetes (CFRD) affects 20% of adolescents and 50% of adults with CF and results in worse lung function and reduced survival. Risk factors for CFRD include the degree of CFTR dysfunction (conferred by disease-causing variants in *CFTR*), other genetic variants known as genetic modifiers, and clinically apparent risk factors such as a history of prediabetic glucose abnormality.

(1) Using data from the CFTR2 project, we show that >99% of individuals with minimal-function (MF) genotypes have developed CFRD by age 60. By comparison, *CFTR* genotypes conferring varying degrees of residual CFTR function reduce the risk of CFRD in a continuous fashion. The impact of highly-effective CFTR modulators on the natural history of CFRD is not yet known, but these data suggest that some type of correction of the CFTR defect could reduce the risk of CFRD, even in people already with some residual CFTR function.

(2) In the CF Genome Project and prior studies, we have identified 20 genetic modifiers which, collectively, alter the risk of CFRD by >4-fold in pwCF with MF genotypes. Much is yet to be discovered about the mechanisms of these diabetes risk variants; some variants also affect the risk of type 2 diabetes in the general population (e.g., *TCF7L2*), other CF complications (e.g., *SLC26A9*), or chronic pancreatitis (*PRSS1*). We are investigating one candidate (*PM20D1*) as a potential biomarker for CFRD.

(3) Genetic modifier studies raise the question as to the overlap of type 2 diabetes and CFRD. Both diseases are caused by progressive insulin deficiency. We will review similarities and differences in pathophysiology and in treatment, and discuss to what extent it is meaningful to diagnose type 2 diabetes in a person with CF.

(4) It may also be possible to gauge the CFRD risk at an individual level. We are combining genetic and non-genetic risk factors (including non-diagnostic measures of dysglycaemia), into a CFRD risk stratification model. This model is able to predict CFRD in a retrospective (pre-modulator) data set (ROC AUC 0.88, further adjustable to achieve high sensitivity or high specificity), and we are collecting prospective data for testing of the model in a current clinical population (CForwaRD study). With improving treatment for CF and increased survival, the prevalence of diabetes is expected to increase, similar to what is seen for type 2 diabetes, also a highly age-dependent form of diabetes. Sufficiently early correction of CFTR dysfunction may be able to reduce the lifetime risk of CFRD, but this potential is yet to be realized, and we already know that even mild forms of CF confer a higher risk of diabetes than in the general population. Therefore, we continue to seek to better understand, diagnose, and treat CFRD across the lifespan.

S5.2 CFRD in adult care / case report demonstrating demands to improve care

Bibi Uhre Nielsen

Cystic Fibrosis Centre Copenhagen, Department of Infectious Diseases, Copenhagen University Hospital – Rigshospitalet, Copenhagen, Denmark

A significant proportion of adults with cystic fibrosis (CF) develop CF-related diabetes (CFRD), which profoundly impacts their quality of life. The fluctuating insulin requirements and frequent hypoglycemic episodes complicate disease management, leading to psychological stress and poor adherence to insulin treatment.

CFRD is a distinct type of diabetes. Due to its rare occurrence in the general population, there is a lack of studies, which hinders the development of evidence-based treatment guidelines. Consequently, CFRD management often relies on experiences from type 1 and type 2 diabetes. Additionally, CFRD is a heterogenous diseases that encompasses a broad spectrum of glucose intolerance. Some individuals experience only short postprandial glucose excursions, while others have fasting hyperglycemia due to minimal endogenous insulin production.

One important clinical question when treating CFRD is when to start insulin treatment, especially for individuals with recent CFRD onset. Early initiation of insulin may preserve beta cell function by alleviating some of the stress on these cells. Insulin treatment has also traditionally been prescribed to people with CF due to its anabolic and pulmonary benefits. However, with the introduction of highly effective modulator therapy, these effects may be less significant. Additionally, the risk of microvascular complications in CFRD appears to be lower compared to other types of diabetes, with some complications only developing in those with fasting hyperglycemia. Nevertheless, early insulin treatment may still offer long-term health benefits.

Another important question when treating CFRD is what can be added when insulin treatment is inadequate. This is particularly relevant for those at the severe end of the CFRD spectrum, who experience prolonged hyperglycemia, possibly due to poor adherence to insulin treatment. In the Danish CFRD population, 10% have an HbA1c > 70 mmol/mol, which is associated with a threefold increase in microvascular complications compared to the target range. This subgroup might benefit from closed-loop systems or oral antidiabetics. However, evidence supporting these treatments is limited and based on small studies. Considering the reduced number of beta cells in CF, treatments that increase insulin secretion could potentially stress and accelerate beta cell deterioration. Another potential strategy is optimizing insulin sensitivity. However, the role of insulin resistance in CFRD is debated, and few trials on insulin-sensitizing drugs have been conducted. Therefore, add-on treatments to standard insulin therapy often rely on the personal experience of diabetologists rather than clinical trial data.

In summary, more questions remain unanswered than answered regarding CFRD management. The introduction of highly effective modulators adds to the complexity, given the longer life expectancy and potential progression of insulin resistance. Sample size remains a challenge in clinical studies, but novel strategies could pave the way for more convenient treatments in the future.

S5.3 Changing nutritional trends in people with CF – what is the impact on management of CF related diabetes?

Joanna Snowball

Over the last decade the nutritional status of people with CF has been changing, this is evidenced by national registry data recording an increased prevalence of those with a body mass index ≥25kg/m2 which would be classified as overweight by WHO classifications (now over 40% in some countries and age groups) and the falling numbers who are underweight BMI >18.5kg/m2 (less than 10% of adults with CF) These trends were present prior to 2019 but have accelerated in the last 5 years in those countries where CFTR modulator use is widespread. The clinical utility of BMI as a marker of health risk is under debate with observational studies showing that body composition, especially lean mass may correlate more closely with lung function. There is concern about the impact of excessive fat mass and visceral adipose tissue on cardiometabolic risk in people with CF. Evidence shows that people with CF often consume a diet that does not meet dietary guidelines for health, this represents the 'CF legacy diet' advice that has traditionally been provided to ensure people with CF met BMI targets linked to improved lung health outcomes. This high fat/high calorie dietary advice with little focus on diet quality has led to ingrained eating habits that are challenging to address. CF diabetes affects around 40% of adults with CF and prevalence increases with age. Types and amount of dietary carbohydrate intake are an important consideration when trying to achieve optimal glucose control. Recent guidelines suggest tailoring dietary advice to BMI and considering the potential impact of excess body weight on the development of insulin resistance. Current research exploring the role of dietary factors in the development of CF related diabetes and the role of low glycaemic index diets in the management of CF related diabetes may lead to a change in guidelines for optimal dietary advice for people with CF.

S5.4 Organ on chip modelling of beta cell function/ pancreatic exocrine-endocrine interface/potential utility of cell therapies

Salem V.[1,2]

[1]Imperial College Healthcare NHS Trust, Diabetes Centre, St Mary's Hospital, London, UK, [2]Department of Bioengineering, Imperial College London, London, UK

Bioengineering approaches for the study and treatment of type 1 diabetes (T1D) have seen huge successes in the past decade. The clinical translation of stem cell derived beta cells to provide a functional cure for T1D is now a reality, providing great hopes for the future although there remain major hurdles to overcome before widespread adoption including post-transplant cell viability and the obviation of systemic immunosuppression . Stem cell derived beta cells also hold hope for the study of particular gene defects or environmental stressors on the risk of diabetes formation. Alongside this, more complex 3D cell cultures, incorporating other elements of islet niche have been developed to study beta cell function in vitro with the aims of enhancing beta cell replacement therapies. In this talk I will cover some of the major recent developments in stem cell derived beta cells and organ on chip technologies as they may apply to the study of cystic fibrosis related diabetes.

29 March — 08:45–10:30

Symposium 06 - Lung regeneration

S6.1 iPSC-derived lung and pancreatic cells: unlocking regenerative therapies for cystic fibrosis

Lalit K. Gautam, Yu Sun, Emma J. Lowden, Denise A. Seabold, Aparna Pathmanathan, Shubha Murthy, Gregory Bonde , Amy L. Ryan

Department of Anatomy and Cell Biology, Carver College of Medicine, University of Iowa, Iowa City, IA, USA

Induced pluripotent stem cell (iPSC) models have become essential tools for studying disease pathogenesis and advancing regenerative medicine, particularly for inaccessible tissues like the airway epithelium and pancreas. iPSC-derived airway and pancreatic progenitor cells offer powerful platforms to investigate disease mechanisms, screen therapeutic interventions, and develop regenerative strategies for cystic fibrosis (CF) and related disorders. By recapitulating key developmental and disease processes in vitro, these models provide critical insights into CF pathophysiology and inform precision medicine approaches.

In the lungs, Highly Effective Modulator Therapy (HEMT) has significantly improved CF outcomes, yet persistent airway inflammation and structural remodeling remain major challenges. Chronic immune activation, disrupted epithelial polarity, and extracellular matrix (ECM) remodeling contribute to excessive mucus production, impaired barrier integrity, and bacterial colonization. While HEMT enhances CFTR function, it does not fully restore epithelial homeostasis, highlighting the need for complementary therapies to mitigate inflammation, enhance mucociliary clearance, and promote epithelial regeneration. Our research leverages iPSC-derived airway epithelial cells to investigate CFTR-dependent epithelial dysfunction and explore the integration of engrafted cells with native airway epithelium.

Beyond the lungs, CFTR dysfunction profoundly impacts pancreatic development and function, leading to exocrine pancreatic insufficiency (EPI) in most individuals with CF. Given the inaccessibility of developing pancreatic tissue, iPSC-derived pancreatic progenitor (PP) cells serve as an invaluable model for studying CFTR's role in early pancreatic specification and exocrine function. Our findings demonstrate that CF-PP cells exhibit impaired differentiation efficiency from the primitive gut tube, a defect recapitulated by pharmacologic CFTR inhibition in non-CF iPSCs. These results suggest a previously unrecognized role for CFTR in pancreatic development and reinforce the potential of iPSC-based regenerative strategies to restore pancreatic function. By integrating iPSC-based lung and pancreas models with multicellular and CF-on-chip functional airway systems, our research provides deeper insights into CF-related epithelial dysfunction and informs regenerative medicine strategies. These studies establish a foundation for developing novel cell-based therapies to improve long-term outcomes for individuals with CF and other epithelial disorders.

S6.2 Transplantation of autologous epithelial cells to airways

Robert E. Hynds

Epithelial Cell Biology in ENT Research (EpiCENTR) Group, Developmental Biology and Cancer Department, Great Ormond Street UCL Institute of Child Health, University College London, London, United Kingdom.

Epidermolysis bullosa (EB) is a group of rare, inherited genetic diseases characterised by epithelial fragility and blistering. Airway involvement in EB is rare, but affected patients experience a high burden of morbidity and mortality. EB airway disease involves granulation tissue formation and scarring of the larynx and trachea. As the disease progresses, airway stenosis (narrowing) necessitates a tracheostomy and can be lethal. LAMA3 variants are common in this patient group, suggesting that LAMA3 variants confer particular susceptibility to airway disease. We have shown that lentiviral expression of wildtype LAMA3A in cultured primary EB patient human airway basal epithelial cells can correct cell adhesion defects (Lau et al., Molecular Therapy, 2024), but it remains unclear how to deliver transduced cells to airways.

To explore stent-based epithelial cell delivery, we developed a surgical transplantation model through short segment tracheal resection and primary anastomosis in New Zealand white rabbits. We establish autologous epithelial cell cultures for each animal following a first surgical procedure. After transduction with a lentivirus encoding ZsGreen and luciferase for cell tracking, cells were seeded onto fibrin sheets and wrapped around bespoke 3D-printed soft elastomer composite tubular constructs. These constructs were delivered to the trachea in a second surgical procedure. Engraftment of transplanted cells was found after 4 or 10 days, demonstrating successful transfer of cells in 13/13 animals. However, after 28 days luciferase signal was significantly reduced, suggesting the loss of transplanted cells over time.

Overall, our data provide initial evidence to support stent-based transplantation of epithelium to the trachea.

S6.3 hiPSC-Derived Airway Epithelium: Exploring CF Physiopathology and Regenerative Strategies.

Marta Vilà-González[1,2,3,4]

[1]Group of Cell Therapy and Tissue Engineering (TERCIT), Research Institute on Health Sciences (IUNICS), University of the Balearic Islands, Palma, Spain , [2]Health Research Institute of the Balearic Islands (IdISBa), Palma, Spain , [3]Department of Fundamental Biology and Health Sciences, University of the Balearic Islands, Spain, [4]Wellcome-MRC Cambridge Stem Cell Institute, University of Cambridge, Cambridge, UK

Human-induced pluripotent stem cells (hiPSCs) have transformed disease modelling and regenerative medicine by enabling the reprogramming of somatic cells into a pluripotent state. These cells possess unlimited expansion potential and can differentiate into virtually any cell type, making them a powerful tool for studying genetic diseases such as cystic fibrosis (CF). Additionally, their amenability to precise genetic modifications allows for the generation of isogenic controls through correction of CFTR mutations in patient-derived cells. This approach not only facilitates disease modelling but also enables the production of autologous, gene-corrected cells with potential applications in cell-based therapies.

We have leveraged hiPSC technology to generate hiPSC-derived airway epithelial cells (hiPSC-AECs) and applied this model to various aspects of CF research. First, we investigated the role of pulmonary ionocytes in the airway epithelium by generating FOXI1 knockout hiPSC-AECs, uncovering novel functions of these cells in epithelial homeostasis. We are also using CF and control hiPSC-AECs to validate insights from publicly available single cell RNA-sequencing datasets, aiming to elucidate how CF affects ionocytes and vice versa. Furthermore, we have explored the engraftment potential of hiPSC-derived airway progenitors in a mouse model of airway injury and are investigating how biomimetic scaffolds could support differentiated hiPSC-AECs in potential cell therapy applications.

Overall, our work demonstrates the versatility of hiPSC-based models for studying airway biology, advancing our understanding of CF pathophysiology, and contributing to potential cell therapies for CF respiratory disease.

P61

S6.4 Mitochondrial metabolic rewiring drives wound healing in cystic fibrosis primary airway epithelium cultures treated with Elexacaftor/Tezacaftor/Ivacaftor

M. Kelly-Aubert[1], E. Bardin[1,2], V. Laigle[3,4,5], A. Deslys[1], A. Hatton[1], B. Chevalier[1], I. Kühl[6], A. Imbard[7], E. Le Guillou[1,7], P. Vargas[1], V. Stoven[3,4,5], I. Sermet-Gaudelus[1,8]

[1]INEM, INSERM U1151, Université Paris Cité, Paris, France, [2]Université Paris-Saclay, UVSQ, INSERM U1173, Montigny le Bretonneux, France, [3]Center for Computational Biology, Mines Paris-PSL, Paris, France, [4]INSERM U900, Paris, France, [5]Institut Curie, Paris, France, [6]Institute of Integrative Biology of the Cell, UMR9198, CEA, CNRS, Université Paris-Saclay, Gif-sur-Yvette, France, [7]Department of Biochemistry, Assistance Publique-Hôpitaux de Paris, Paris, France, [8]Service de Pneumologie et Allergologie Pédiatriques Centre de Référence Maladies Rares, Mucoviscidose et maladies de CFTR, Hôpital Necker Enfants Malades, Paris, France

CFTR modulators have changed the prognosis and quality of life of patients, but their exact mechanism of action remains incompletely understood. A multi-omic study in primary nasal epithelial cells from F508del children highlighted mitochondria-related pathways as being an important target of Elexacaftor/Tezacaftor/Ivacaftor (ETI). The present study analyzes the impact of ETI on several key mitochondrial pathways in F508del primary cultures. ETI induced an increase in mitochondrial mass as shown by a significant increase in both TOMM20 immunostaining and mitochondrial DNA to nuclear DNA ratio. This was concomitant with a significant increase in *PGC1a* and *TFAM* mRNA expression, key regulators of mitochondrial biogenesis. Mitochondrial activity assessed by oxygen consumption rates (OCR) in Seahorse assays revealed that ETI restored deficient maximal OCR and spare respiratory capacity in F508del cultures to WT levels.
We next assessed fatty acid oxidation flux in F508del cultures by administering deuterated palmitate and quantifying the downstream fatty acids. This highlighted an increase in fatty acid oxidation flux upon ETI as well as a significant increase in the expression of the fatty acid oxidation rate-limiting enzyme CPT1. As fatty acid metabolism is essential for airway epithelium repair[1] , we performed wound healing assays and demonstrated that F508del cells have a lower wound healing rate than WT cells and that this is restored with ETI. The addition of the CPT1 inhibitor, Etomoxir, inhibited fatty acid oxidation and abolished the effect of ETI on wound healing rates. This suggests that ETI induces mitochondrial metabolic rewiring involving oxidative phosphorylation and fatty acid oxidation and that this participates in enhanced airway epithelium repair capacities highlighting a new role of CFTR in airway epithelial repair and metabolic pathways.

1. Crotta, S., Villa, M., Major, J. et al. Repair of airway epithelia requires metabolic rewiring towards fatty acid oxidation. Nat Commun 14, 721 (2023)

P58

S6.5 Unveiling new insights of ionocytes' role in cystic fibrosis using single-cell RNA sequencing analysis and human induced pluripotent stem cell-derived airway epithelial cells

M. Salvà-Barceló[1,2], J. Muncunill-Farreny[2], L. Vallier[3,4], J.M. Ramis[1,2,5], M. Monjo[1,2,5], M. Vilà-González[1,2,5]

[1]University of the Balearic Islands, Group of Cell Therapy and Tissue Engineering (TERCIT) - Research Institute on Health Sciences (IUNICS), Palma de Mallorca, Spain, [2]Health Research Institute of the Balearic Islands (IdISBa), Palma de Mallorca, Spain, [3]Berlin Institute of Health (BIH) at Charité, BIH Center for Regenerative Therapies, Berlin, Germany, [4]Max Planck Institute for Molecular Genetics, Berlin, Germany, [5]University of the Balearic Islands, Departament of Fundamental Biology and Health Sciences, Palma de Mallorca, Spain

Pulmonary ionocytes are a rare airway epithelial cell (AEC) type expressing high levels of the cystic fibrosis transmembrane conductance regulator (CFTR). CFTR is an ion transport channel whose loss of function mutations are the underlying cause of cystic fibrosis (CF) disease. Thus, ionocytes have been hypothesised to play a key role in the pathophysiology of CF.

Even though single-cell RNA sequencing (scRNA-seq) technology has enabled the detailed transcriptomic profiling of AECs, including ionocytes, the function and disease-specific transcriptomic changes of these cells are still unclear. To address this question, we have explored publicly available scRNA-seq data from air-liquid interface (ALI) cultures derived from primary bronchial epithelial cells aiming to identify transcriptomic disease-specific changes between ionocyte populations from healthy individuals (control or CO) and from CF patients (Carraro *et al.*, 2021). Our analysis has revealed more than 10 genes that are differentially expressed in CF vs CO ionocytes. Additionally, gene set enrichment analysis has indicated transcriptomic changes related to biological processes such as regulation of immune response, oxidative phosphorylation and cilia movement.

However, these findings are based on transcriptomic data of samples with different genetic backgrounds and require to be validated using isogenic *in vitro* models. The study of ionocytes is restricted due to the limited availability of primary tissue and the lack of appropriate models. Human induced pluripotent stem cells (hiPSC) could be a promising approach as they can be expanded indefinitely and can be differentiated providing an unlimited source of AECs. Moreover, hiPSCs can be easily genetically modified to correct CFTR mutations to generate isogenic controls from patient-derived cells. Nevertheless, their differentiation into AECs can be challenging as there is a lack of standardized protocols and most of them have failed to consistently produce rare AECs such as ionocytes. In previous studies we have successfully developed an *in vitro* airway model using hiPSC lines from CF patients and isogenic counterparts (wildtype or WT) that offers a platform to study ionocytes. Isogenic CF and WT lines were parallelly differentiated using our previously described protocol (Vilà-González *et al.*, 2024) to generate an isogenic hiPSC-AEC *in vitro* model to validate our transcriptomic findings. After 28 days of AEC maturation in ALI culture, airway epithelium was characterised through different molecular biology techniques including immunofluorescent staining. We have confirmed the presence of abundant AEC types (basal, goblet and secretory cells) along with less frequent cell types such as ionocytes and pulmonary neuroendocrine cells (PNECs), which show characteristic morphology. Moreover, we have confirmed the expression of key genes of interest from our transcriptomic analysis in our isogenic *in vitro* model.

Our hiPSC-AEC isogenic *in vitro* model contains both abundant and rare AECs and has allowed us to validate some of our transcriptomic findings. This model also has the potential to be used to study other aspects of CF and other respiratory diseases. Furthermore, our findings provide novel insights into the potential role of ionocytes in CF pathophysiology, bringing us a step closer to identifying new therapeutic approaches.

P59

S6.6 GY971, a new anti-inflammatory agent for the treatment of Cystic Fibrosis lung disease

I. Lampronti[1,2], C. Tupini[1], S. Fagnani[1], G. Raso[1], A. Chilin[3], G. Marzaro[3], A. Tamanini[4], N. Pedemonte[5], V. Capurro[5], O. Laselva[6], C. Allegretta[6], A. Bragonzi[7], G. Cabrini[1,2]

[1]University of Ferrara, Department of Life Sciences and Biotechnology, Ferrara, Italy, [2]University of Ferrara, Center of Innovative Therapies for Cystic Fibrosis (InnThera4CF), Ferrara, Italy, [3]University of Padova, Department of Pharmaceutical and Pharmacological Sciences, Padova, Italy, [4]University of Verona, Department of Engineering for Innovation Medicine, Verona, Italy, [5]IRCCS Istituto Giannina Gaslini, U.O.C. Medical Genetics, Genova, Italy, [6]University of Foggia, Department of Clinical and Experimental Medicine, Foggia, Italy, [7]IRCCS San Raffaele Scientific Institute, Infections and Cystic Fibrosis Unit, Milan, Italy

In *Cystic Fibrosis* (CF), chronic lung inflammation and progressive pulmonary damage persist despite the use of *highly effective CFTR modulator therapy* (HEMT). For this reason, there is an urgent need for innovative anti-inflammatory agents to be administered via the pulmonary route to people with CF (pwCF), working through novel mechanisms and maintaining an acceptable side effect profile. GY971 is a new synthetic furocoumarin derivative developed to inhibit NF-kB/DNA complexes and characterized by anti-inflammatory action, at nanomolar concentrations, based on the regulation of neutrophil chemotaxis mediators in CF bronchial epithelial cells *in vitro* and murine lungs *in vivo*. The study was focused on the analysis of GY971 in CF bronchial epithelial cell lines exposed to *P.aeruginosa* (PAO-1) or TNF-a *in vitro*, and in mouse models of *P.aeruginosa* lung infection *in vivo*. The anti-inflammatory effect of GY971 was also validated in primary HBE (Human Bronchial Epithelial) cells and HNE (Human Nasale Epithelial) cells derived from pwCF.
Thanks to the promising results obtained, GY971 was recently approved by the *European Medicines Agency* (EMA) as orphan drug for CF. Ongoing research aims to consolidate its efficacy and safety profile, bringing it closer to clinical application as an innovative anti-inflammatory treatment for CF lung disease.

29 March — 11:00–12:45

Symposium 07 - Gut microbiome, gut-lung axis

S7.1 Dyslipidemia impacts host defense and chronic infections in the CF airways through the gut-lung axis

Rebecca Valls[1], Catherine Armbruster[1], Rachel Willis[2], Nikolaos Dedousis[2], Vaughn Cooper[3], Alison Kohan[2], Jennifer Bomberger[1]

[1]Geisel School of Medicine at Dartmouth, Hanover New Hampshire USA, [2]University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania USA, [3]University of Pittsburgh, Pittsburgh, Pennsylvania USA

Emerging evidence supports a gut-lung axis in CF, where nutrients from the gastrointestinal (GI) tract and GI-resident microbes can regulate lung disease and respiratory infections in CF. Gut microbiome dysbiosis in people with cystic fibrosis (pwCF) begins in early childhood, marked by reduced microbial diversity, delayed microbiome maturation, and a shift in community structure. Dysbiosis in pwCF is driven by CFTR mutations impacting mucus, inflammation, gut motility and diet. Dietary fat is absorbed in the small intestine, packaged in chylomicrons, and secreted across the intestine directly into the mesenteric lymph prior to entering the blood at the left subclavian vein. Because of this unique anatomic arrangement, chylomicrons are first delivered to the heart and lungs. Malabsorption of nutrients vis this pathway is has been linked to *Pseudomonas aeruginosa (Pa)* lung infections and all-cause mortality in pwCF. A major gap in the field, even with modulator therapy, is determining how CF-driven defects in the intestine regulate the events in the lung. Using a specialized surgical model to directly measure dietary lipid absorption kinetics from $CFTR^{-/-}$ mice, we have observed a critical defect: CFTR deficiency inhibits dietary fat absorption into lymph, deters fatty acid trafficking across the apical GI epithelial membrane, and results in the secretion of defective chylomicrons in response to dietary fat. These observations are supported by clinical studies in pwCF. To examine defects in respiratory function due to defective chylomicrons in CF, we have developed an in vitro model where human CF airway epithelial cells (CF AECs) are exposed basolaterally to chylomicrons. We observe that CF AECs express fatty acid transporters and basolateral chylomicron treatment of CF AECs results in apical airway lipid accumulation. To examine how dyslipidemia impacts respiratory infections with *Pa*, we performed an experimental evolution study of *Pa* evolved with chylomicron-exposed CF AECs. Experimental evolution of *Pa* in association with chylomicron-treated AECs selects for *Pa* variants in acyl transferase and dehydrogenase genes, which include enzymes in fatty acid metabolism (Fad pathway). Fad mutations have been shown to enhance *Pa* resistance to envelope stress, including antimicrobial treatments and phagocytic clearance. In fact, we observe *Pa* mutants in the Fad pathway have increased resistance to tobramycin, as well as macrophage-mediated killing. Interestingly, similar variants are observed in microbial evolution studies of *Pa* from pwCF on HEMT, providing evidence of the gut-lung axis in pwCF that is changing the HEMT era.

S7.2 Impacts of CFTR modulator therapies on the gut microbiome

Christopher van der Gast

Northumbria University, Newcastle, UK

People with CF (pwCF) suffer from persistent gastrointestinal (GI) symptoms and abnormalities that impact quality of life. Perhaps unsurprisingly, the research question "*how can we relieve GI symptoms, such as stomach pain, bloating, and nausea?*" remains a top priority for clinical research in CF. The CF gut is characterized, both directly and indirectly due to CF transmembrane conductance regulator (CFTR) dysfunction, as having excess mucus, increased fat content, acidic pH, increased inflammation, and increased antibiotic perturbation. Also present with GI abnormalities is a disturbance or dysbiosis of the gut microbiome, which are changes to the resident microbiota and their functional outputs that are hypothesized to exacerbate abnormalities associated with CFTR dysfunction. This is concerning as it is now established that the gut microbiome is central to human nutrition and health. CFTR modulator therapies are now in widespread use and are reportedly having profound positive effects for most pwCF. However, there is a dearth of knowledge on the longer-term effects of CFTR modulator therapies upon the gut microbiome and associated outcomes. Our work has found that Tezacaftor/Ivacaftor therapy had negligible effects on the CF gut microbiome. Conversely, when we investigated the impact of extended Elexacaftor/Tezacaftor/Ivacaftor therapy, we observed a shift in the CF gut microbiota towards compositions in healthy controls. However, significant differences persisted between CF and healthy control microbiome characteristics. We posit that respiratory antibiotics likely hinder CF gut microbiota shifting to a healthy state.

S7.3 Lessons from the gut-lung axis in the CFTR modulator era

Laurence Delhaes, Florian Lussac-Sorton, Sébastien Imbert, Raphael Enaud

Inserm U1045 & Bordeaux CHU, Bordeaux, FRANCE

The advent of CFTR modulators represents a turning point in the history of cystic fibrosis management, changing profoundly the disease's clinical course by improving mucosal hydration. CFTR modulators are known to improve chloride channel function, consequently modifying microbiome of the lungs and the gut. While the gut-lung axis is established in other settings, little is known about its role following modulator therapy, particularly in the 2-11 age group. Therefore, assessing bacterial and fungal microbiome changes in the airways and digestive tracts is of great interest to better understand the mechanisms and to predict disease evolution.

The bacterial and fungal dysbiosis have been well documented in cystic fibrosis patients; yet the impact of CFTR modulators on microbial communities especially regarding the gut-lung axis has been partially deciphered to date. Recently, we investigated the effects of lumacaftor-ivacaftor on the bacterial and fungal microbiome of the gut-lung axis in a national multicenter pediatric study. Notably, two lung microbiota response profiles emerged: "responders" showed increased lung bacterial diversity and reduced inflammation, whereas "non-responders" exhibited minimal changes. Gut and lung microbiome composition at baseline predicted these distinct trajectories with 81% accuracy, using random forest model. Microbial interactions within the gut-lung axis peaked at 3 months post-initiation; same approaches are ongoing in the national cohort treated with elexacaftor-tezacaftor-ivacaftor ("Kaf-Biota" project). These data along with published ones will be reviewed and discussed in a gut-lung axis perspective, in order to decipher the gut-lung axis involvement in cystic fibrosis pathophysiology and its evolution in the era of novel modulators therapies.

P65

S7.4 The gut's role in pulmonary and extrapulmonary manifestation of Cystic Fibrosis

C. Cigana[1], G. Delmonte[1], A. Facoetti[2], L. Veschetti[1], F. Gianferro[1], A. Neri[1], R. Fiorotto[3], A. Livraghi-Butrico[4], L. Massimino[2], F. Ungaro[2], A. Bragonzi[1]

[1]San Raffaele Scientific Institute, Division of Immunology, Transplantation, and Infectious Diseases, Milano, Italy, [2]San Raffaele Scientific Institute, Division of Immunology, Transplantation, and Infectious Diseases, Experimental Gastroenterology, Milano, Italy, [3] Yale School of Medicine, Department of Internal Medicine, Section of Digestive Diseases, Yale Liver Center, New Haven, United States, [4]The University of North Carolina, Cystic Fibrosis/Pulmonary Research and Treatment Center, Chapel Hill, United States

Introduction: Cystic Fibrosis (CF) research primarily focuses on the lung, limiting understanding of disease pathogenesis and chronic infections in pwCF, including those on CFTR-modulator treatment. This gap may result from insufficient studies on distal organs.
Objectives: We developed a new mouse model of CF with deltaF508-Cftr mutation into genetically diverse Collaborative Cross (CC) background (CC037 HO). The aim was to have a suitable genetic background for CF, uncover novel pathological mechanisms, and explore the role of distal organs beyond the lung.
Methods: Microbiological and inflammatory responses were characterized by standard techniques (bacterial culture, FACS, and immunostaining), as well as genomic tools (metatranscriptomics and single-nucleus RNA-seq).
Results: Early-life respiratory inflammatory profiles in the lung and blood were evident in CC037 HO mice. Sn-RNA-seq showed upregulated immune response pathways, along with enhanced defense responses to bacteria. Microbiological cultures showed enteric bacteria, with *Escherichia coli*, *Enterococcus faecalis*, and *Klebsiella oxytoca* being the most prevalent in the lung of CC037 HO but absent in WT mice. Metatranscriptomics revealed shared microbiota between the colons and lungs of CC037 HO mice, whereas CC037 WT mice showed distinct microbiota. Similar enteric bacteria were found in the livers of CC037 HO mice, where inflammation was detected. Gut of CC037 HO mice was characterized by mucus accumulation, inflammatory responses, and a higher bacterial load in mucosa and stool compared to WT mice. This was associated with compromised barrier integrity and increased gut permeability, enabling potential organ access. Treatment with laxative rescued gut barrier integrity, and respiratory and systemic inflammation in CC037 HO mice.
Conclusion: Our data support the role of the gut in systemic effects, with potential impacts on distal organs such as the lungs and liver.

Supported by Italian CF Research Foundation and CF Foundation.

P64

S7.5 Gut microbiota-metabolome relationships across pwCF taking highly effective CFTR modulators: Preliminary findings from the GRAMPUS-CF study

R. Marsh[1], W. Cheung[1], D. Arends[1], D. Sills[2,3], D.W Rivett[4], A.R Smyth[2,3,5], C. van der Gast

[1]Northumbria University, Department of Applied Sciences, Newcastle upon Tyne, United Kingdom, [2]University of Nottingham, Academic Unit of Lifespan & Population Health, School of Medicine, Nottingham, United Kingdom, [3]NIHR Nottingham Biomedical Research Centre, Nottingham, United Kingdom, [4]Manchester Metropolitan University, Department of Natural Sciences, Manchester, United Kingdom, [5]Queen's University Belfast, School of Medicine, Dentistry and Biomedical Sciences, Belfast, United Kingdom

Background: Relationships between gut microbiome structure, intestinal physiology, and wider gastrointestinal symptoms are yet to be fully elucidated within cystic fibrosis. Despite shifts to highly effective CFTR modulators, people with cystic fibrosis (pwCF) still experience negative intestinal symptoms and exhibit signs of a general gut microbiota dysbiosis as compared to controls from the wider population. Further understanding of disease interplay is therefore warranted at the intestinal site, particularly to support the potential development of additional interventions, therapeutics, and clinical practices to relieve morbidity across pwCF.
Aims: Baseline faecal samples across children and adults from the Gut Research Advancing a Mechanistic and Personalised Understanding of Symptoms in Cystic Fibrosis (GRAMPUS-CF) study were utilised to investigate relationships between microbiota composition and the wider intestinal metabolome.
Methods: Gut microbiota profile was determined using PacBio full-length 16S HiFi sequencing (n = 70). Untargeted metabolomics, including additional lipidomics, were performed with ultra-high-performance liquid chromatography high-resolution mass spectrometry (UHPLC-HRMS) (n = 64). Correlation-based approaches were used to construct networks highlighting relationships between taxa and metabolites across paired samples Participant clinical data was further integrated to investigate potential associations with microbiota structure and function under treatment with elexacaftor/tezacaftor/ivacaftor**.**
Results: From our preliminary analyses, we confidently identified a wide array of unique metabolites from the metabolomic (*n* = 660) and lipidomic approaches (*n* = 527). Network analyses highlighted both positive and negative associations across beneficial and potentially pathogenic taxa, extending to relationships with metabolites and lipids of physiological interest in the CF intestine. pwCF clinical demographics also influenced the microbiota, metabolome and lipidome.
Conclusions: Faecal multi-omics indicate important relationships with gut microbiota structure across pwCF undertaking CFTR modulator therapy. Temporal dynamics of the intestinal microbiome will be investigated across elexacaftor/tezacaftor/ivacaftor (ETI) therapy in the GRAMPUS-CF cohort. Further clinical data and intestinal physiology metrics will be available to aid our analyses. This also includes the integration of participant intestinal symptom data.
Acknowledgements: Many thanks to the Cystic Fibrosis Trust for funding this work (SRC 023). Most importantly we would like to thank all the participants have enrolled onto GRAMPUS-CF. Without them this research would not be possible.

P63

S7.6 Modeling a Triple Culture Airway-on-a-Chip for Inflammation Studies in Cystic Fibrosis

Y. Man[1,2], A. Jiang[1,2], A. Stejskalová[1], H. Bai[3], G. Di Bonaventura[4], M. Romano[4], D. Ingber[1,2,5], R. Plebani[4]

[1]Wyss Institute for Biologically Inspired Engineering - Harvard University, Boston, United States, [2]Boston Children's Hospital - Harvard University, Boston, United States, [3]Xellar Biosystems, Boston, United States, [4]"G. d'Annunzio" University of Chieti-Pescara, Department of Medical, Oral and Biotechnological Sciences, Chieti, Italy, [5]Harvard John A. Paulson School of Engineering and Applied Sciences - Harvard University, Cambridge, United States

Background: Cystic fibrosis (CF) is characterized by chronic lung inflammation and uncontrolled polymorphonuclear leukocyte (PMN) migration. We developed the first CF Airway-on-a-Chip model, composed of CF human bronchial epithelial cells (HBEs) and non-CF human vascular endothelial cells (HVECs), demonstrating increased inflammation and PMN migration compared to a non-CF chip. However, it is clear that HVECs and fibroblasts play a relevant role in CF airway inflammation. Thus, an airway chip built with all CF cellular components will be fully representative of a CF airway unit, offering a high-fidelity human CF preclinical model suitable for disease modeling and pharmacological studies.

Aims: Aim of this study was to develop the first CF triple culture Airway-on-a-Chip prototype in a microfluidic device composed of CF-HBEs, CF-HVECs, and CF stroma (CF Airway-on-a-Chip 2.0).

Methods: CF and non-CF HBEs, HVECs and fibroblasts were cultured in a two-channel microfluidic chip. Cells were characterized by immunocytochemistry and confocal imaging. Cytokine levels were measured by Luminex® and analyzed with the Prism GraphPad software.

Results: We established the first CF triple culture in a microfluidic device with differentiated epithelium containing ciliated, goblet, club, and basal cells in the upper channel, and fibroblasts and endothelial cells in the basal channel. Preliminary experiment comparing CF Airway-on-a-Chip 2.0 to a control chip with non-CF cells revealed increased PMN recruitment, with the endothelium playing a central role. The CF culture uniquely secreted detectable P-selectin and exhibited higher TNF-α levels than the non-CF counterpart. Moreover, CF-HVECs cultured alone released higher ICAM-1, TNF-α, IL-8, IL-6, GM-CSF, and MIP-1α levels compared to non-CF HVECs, alongside a decrease in MCP-1 levels. This correlated with increased PMN adhesion.

Conclusions: The CF Airway-on-a-Chip 2.0 provides an implemented version of the previous model, enabling the study of additional CF disease features as stromal remodeling and endothelial dysfunction. It highlights the pivotal role of endothelial cells in CF inflammation, especially in neutrophil recruitment, thus offering a valuable platform for exploring new treatments targeting CF inflammation.

29 March — 14:15–16:00

Symposium 08 - Gene editing and delivery

S8.1 Systemic in utero gene editing as a treatment for cystic fibrosis

Adele S. Ricciardi, Christina Barone, Rachael Putman, Elias Quijano, Anisha Gupta, Richard Nguyen, Hanna Mandl, Alexandra Piotrowski-Daspit, Francesc Lopez-Giraldez, Valerie Luks, Mollie R. Freedman-Weiss, James Farrelly, Samantha Ahle, Peter M. Glazer, W. Mark Saltzman, David H. Stitelman, Marie E. Egan

Yale University, New Haven, CT, USA

Cystic fibrosis (CF), a monogenic disease resulting from mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene, affects multiple organs, including the respiratory, gastrointestinal, and reproductive systems. Irreversible pathology in these tissues is often already present at the time of birth, suggesting that a true curative therapy would require intervention during fetal development. Treating patients with CF early is crucial for preventing or delaying irreversible organ damage. We tested if triplex forming peptide nucleic acids (PNAs) encapsulated in polymeric nanoparticles and delivered systemically in utero could result in multiorgan mutation correction in a CF mouse model.

First, we evaluated the cellular tropism of polymeric nanoparticle (NP) administration to the mid-gestation murine fetal lung using two delivery approaches: intravenous (i.e., systemic), via vitelline vein injection, and direct instillation into the amniotic fluid of dye loaded nanoparticles. Systemic delivery resulted in more robust NP accumulation in the fetal lung, including in pulmonary epithelial cells. Subsequently, we investigated if systemic NP delivery would result in higher levels of gene editing in the fetal lung when compared to intra-amniotic delivery in a GFP reporter model. In these experiments, gene editing is achieved via delivery of triplex forming peptic nucleic acids (PNAs) and donor DNA encapsulated in polymeric nanoparticles.

Next, we examined if in utero treatment with NPs containing a PNA/donor DNA pair designed to correct the most common disease-causing CFTR mutation (F508del) results in gene correction in multiple tissue types affected by CF (lung, pancreas, gut) in a CF mouse model.

We found that in utero editing was associated with sustained postnatal CFTR activity in both respiratory and gastrointestinal tissue, without detection of off-target mutations in partially homologous loci. Importantly, we find that the sustained CFTR activity associated with gene correction was present well into adulthood of the mouse, after a single fetal treatment. This work suggests that systemic in utero gene editing may represent a viable strategy for treating monogenic diseases before birth that impact multiple tissue types.

S8.2 Artificial intelligence-guided design of lipid nanoparticles for cystic fibrosis gene therapy

Jacob Witten[1], Idris Raji[1], Rajith S Manan[1], Emily Beyer[1], Sandra Bartlett[1], Yinghua Tang[2], Mehrnoosh Ebadi[2], Junying Lei[2], Dien Nguyen[1], Favour Oladimeji[1], Allen Yujie Jiang[1], Elise MacDonald[1], Yizong Hu[1], Haseeb Mughal[1], Ava Self[1], Evan Collins[1], Ziying Yan[2], John F Engelhardt[2], Robert Langer[1], Daniel G Anderson[1]

[1]Massachusetts Institute of Technology, Massachusetts, USA, [2]University of Iowa, Iowa, USA

Ionizable lipids are a major component of lipid nanoparticles (LNPs), a leading nonviral messenger RNA (mRNA) delivery technology. Here, we introduce **Li**pid **O**ptimization using **N**eural networks (LiON), a deep-learning strategy for ionizable lipid design. To train LiON, we created a dataset of >9,000 lipid nanoparticle activity measurements and fed this data to a directed message-passing neural network for prediction of nucleic acid delivery with diverse lipid structures. Lipid optimization using LiON successfully predicted RNA delivery for both *in vitro* and *in vivo* held-out test sets and extrapolated to structures divergent from the training set. We next evaluated 1.6 million lipids *in silico* and identified two structures, FO-32 and FO-35, with state-of-the-art local mRNA delivery to the mouse muscle and nasal mucosa. FO-32 also matched the state of the art for nebulized mRNA delivery to the mouse lung, and both FO-32 and FO-35 efficiently delivered mRNA to ferret lungs, representing the first published example of mRNA delivery to ferret conducting airways. Overall, this work shows the usefulness of deep learning for improving nanoparticle delivery and introduces LNPs with promising activity for cystic fibrosis treatment.

S8.3 Development of mRNA and ribonucleoprotein delivery tools for lung targeted gene therapy in cystic fibrosis

G. Maule[1], S. Saxena[2], A.Collliva[2], S. Vodret[2], J. Parot[3], A. Molska[4], E. Gurrieri[1], M. Stancampiano[1], D. Guidone[5], A. Borrelli[5], S.E. Borgos[3], L. J. V. Galietta[5], S.Hak[3], S. Zacchigna[2], F. Zerbini[1], A. Cereseto[1]

[1]Department CIBIO, University of Trento, Trento, Italy, [2]International Centre for Genetic Engineering and Biotechnology, Cardiovascular Biology Laboratory, Trieste, Italy, [3]SINTEF, Department of Biotechnology and Nanomedicine, Trondheim, Norway, [4] SINTEF, Trondheim, Norway, [5]Telethon Institute of Genetics and Medicine, TIGEM, 80078 Pozzuoli, Italy

Genome editing technologies hold great potentials for the treatment of cystic fibrosis (CF). However, current delivery methods remain inefficient, posing a major barrier to their clinical translation.

The GenDel-CF project aims to develop in vivo delivery tools to transfer 1) mRNA and 2) ribonucleoprotein (RNP) expressing CRISPR-Cas9.

For the first goal our consortium aims to develop novel lipid nanoparticle (LNP) formulations. We recently developed a specific lipid formulation (LNP) which showed efficient mRNA delivery via intra tracheal (IT) injection. The LNP were tested by encapsulating Cre-mRNA and delivered to CRE-lox reporter mice. We obtained high levels of Cre-mediated recombination resulting in mGFP expression throughout the lung parenchyma upon both administration routes. IT administration resulted nearly 80% of GFP-positive in secretory cells, 48% in epithelial cells from the airways and submucosal glands, and 75% in bronchial epithelial and alveolar type 1 cells. This pointed to IT delivery as the best delivery route for targeting epithelial cells critical for CF therapy.

For the second aim, consisting in CRISPR-Cas delivery as RNP we focused on engineered vesicles as promising delivery systems for genome editing. We exploited base editors as editing systems, specifically ABE8e-SpCas9, due to their proven efficiency in precisely and effectively repairing CFTR mutations. The GE-vesicles were produced using membrane anchoring motifs to capture maximal amounts of ABE8e-SpCas9 and sgRNA. Particles were characterized for size, particle number and editor content showing overall homogenous size in the range of 100-150 nm and efficient encapsulation of the ABE8e-SpCas9 resulting in up to 60% of base conversion.

In conclusion, our LNP formulation and GE-vesicles lay the foundation for editing tools delivery to repair CF mutations.

P74

S8.4 Journey to the Centre of the Airway: tackling cystic fibrosis with cell-penetrating VP22-ABEs

J. Alves[1,2,3], C. M. Farinha[2], P. T. Harrison[3]

[1]Biosciences Institute, University College of Cork, Department of Physiology, Cork, Ireland, [2]BioISI-Biosystems and Integrative Sciences Institute, Faculty of Sciences, University of Lisbon, Lisbon, Portugal, [3]Division of Pulmonary Medicine, Cincinnati Children's Hospital, Cincinnati, OH, United States

Gene editing targeting the progenitor lung basal cells (BC) may have the potential to repopulate the lung pseudostratified epithelium (LPE) with cells expressing a functional CFTR protein for any CF-causing mutation. Whilst luminal epithelial cells (LEC) can be readily transfected with DNA or RNA molecules encoding gene-editing ribonucleoprotein (RNP) complexes, the complex architecture of the LPE causes a potential impediment to the direct targeting of BC. Considering this, we wanted to determine if DNA or RNA molecules delivered to the LEC could transfer their gene-editing encoded RNPs to the nuclei of BC cells below.

Inspired by a previous study using the HSV-1 VP22 cell-penetrating peptide[1], we developed VP22-GFP and VP22-adenine base editor (VP22-ABEs) fusion proteins. We hypothesise that VP22-ABE-RNPs may retain VP22's intrinsic nuclear localization and intercellular trafficking capacity, thus being capable of traveling through the LPE and successfully delivering gene-editing RNPs to BC.

Expression vectors encoding VP22-GFP, VP22-ABE8e(NG), and VP22-ABE9-SpRY, were designed using gBlocks containing a CMV promotor. Initial characterisation of pVP22-GFP in HEK293T cells showed high levels of GFP expression 48h post-transfection and a distinct nuclear localisation pattern relative to the pGFP control, confirming the nuclear localisation capacity of VP22-GFP fusion proteins.

The base editing profile and editing window of VP22-ABEs and parental ABEs was characterised using 6 different gRNAs targeted to regions of the human genome present in HEK293T cells. The editing window of ABE8e(NG) and VP22-ABE8e(NG) covers positions 2 to 12 in the spacer region; the highest editing levels achieved at adenine in position 5 (A5) with 76.0 and 64.4%, respectively, and moderate levels in positions A3 to A7. Differently, the editing window of SpRY-ABE9 and VP22-SpRY-ABE9 only includes positions 5 to 6 in the spacer region; with slightly lower editing levels achieved at A5 with 53.8 and 45.7%, respectively, yet with a positive pay-off of minimal levels of editing at A6.

To explore the intercellular trafficking capacity of VP22-ABEs between co-culture models, we developed a mCherry-Q47X fluorescence reporter cell model. We will electroporate HEK293T cells with VP22-ABEs and a gRNA-specific for the PTC in the mCherry-Q47X reporter. Co-culture of electroporated HEK293T cells and untransfected mCherry-Q47X will allows evaluation of our hypothesis that the VP22-ABE-gRNA-RNP complex can shuttle from the producer HEK293T cells into the neighbouring mCherry-Q47X reporter cell line and edit the PTC, thus demonstrating that VP22's intrinsic intercellular trafficking activity can shuttle ABE-gRNA complexes. Moreover, we will assess the VP22-ABEs intercellular trafficking and editing capacity in differentiated air-liquid interface (ALI) cultures, such as the BCi-NS1.1 with the W1282X variant which we have shown can be fully-differentiated into different cell types of the LPE. We will determine if VP22-ABE-RNP complexes produced in LEC can shuttle to the nuclei of BC in this model and functionally correct the W1282X variant using fluorescence-activated cell sorting to select for $NGFR^{+ve}$ BC; a substantial difference between the levels of VP22-ABEs *versus* parental-ABEs between the entire pool of cells and BC cells only, would be indicative of successful shuttling and editing capacity of VP22-ABE-RNP complexes into BC.

[1]PMID:10341888

P67

S8.5 Development of a novel Adenine Base Editor with temporospatial control of the editing

I. Zollo[1], L. Santos[1,2,3], J. Alves[1,4], A.S Coroadinha[2,3], P.T Harrison[4,5], C.M Farinha[1]

[1]BioISI - Biosystems and Integrative Sciences Institute, Faculty of Sciences, University of Lisboa, Lisbon, Portugal, [2]iBET, Instituto de Biologia Experimental e Tecnológica, Oeiras, Portugal, [3]Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras, Portugal, [4]Physiology Department, University College Cork, Cork, Ireland, [5]Cincinnati Children's Hospital, Cincinnati, OH, United States

Despite the great advances witnessed with the approval of CFTR modulators, 10-15% of people with cystic fibrosis (pwCF) still lack a causal treatment, namely those with nonsense variants that abolish protein synthesis. In these cases, gene-based therapies represent the best possibility for a cure.
Among all genome editing techniques available, Adenine Base Editor (ABE) is one of the most promising as it uses a Cas9 nickase (nCas9) fused to a TadA enzyme that can convert any adenine (A) in the editing window into guanine (G), being therefore suitable for the correction of W1282X mutation, the second most common CF-causing variant lacking a therapy.
Besides the development of an effective gene editing tool, it is not clear yet which cell types in the pseudostratified lung epithelium (and how many of them) need to be edited to restore CFTR function to levels that correspond to clinical benefit.

To find an answer to these fundamental questions, we developed a basal cell line bearing the nonsense W1282X mutation (BCi W1282X-CFTR) that retains the ability to differentiate into all the cell types of the airway epithelium and a new CRISPR-based tool, which is a split version of ABE (SplitABE), that allows temporal control of the editing. Our preliminary results show that SplitABE is able to reverse the premature stop codon (TGA) back to the WT (TGG) and the temporal control of the editing reduces the levels of bystander edits, usually associated with ABE[1].
Here, we now report a strategy to couple the temporal regulation of the ABE with spatial control to allow us to tackle this question. To do this, we cloned the SplitABE cassette under the control of five different cell-type specific promoters, covering the main cell types in the airway epithelium (basal, ciliated, secretory type 1 and 2, ionocytes)[2].

The five final plasmids obtained were used to transfect the packaging cell line 293T to produce lentiviral particles on a large-scale. To determine lentiviruses infectious titers, the BCi W1282X-CFTR cell line was transduced with different dilutions of the viruses and the DNA was extracted after 72h. The absolute amount of target DNA (viral gene) was quantified by Droplet Digital PCR (ddPCR).
Taking into consideration the number of transduced cells, the volume and the dilution of the viruses, we calculated an LV titer between 7E+06 and 1,5E+07 IU/mL for the different viruses.
According to these quantifications, we are currently transducing the BCi W1282X-CFTR cells with the LV at various MOIs to generate novel cell lines with cell type-specific expression of the SplitABE.

In conclusion, this novel gene editing tool combined with the newly generated cell model will allow us to identify

1. Santos L et al (2023) J Cyst Fibr 22 (S2), S32.
2. Zollo I et al (2024) J Cyst Fibr 23 (S2), S162.

Acknowledgements: Work supported by grants HARRIS21G0 and FARINH24G0 from CFF and centre grants UIDP/04046/2020 and UIDB/04046/2020 (doi: 10.54499/UIDB/04046/2020) from FCT, Portugal (to BioISI).

P69

S8.6 Precise correction of challenging mutations causing cystic fibrosis through transient delivery of the prime editing machinery

M. Bulcaen[1], L.L. Dipalo[1,2], B. Poelmans[2], S. Thierie[2], M. Smits[1], M. De Roy[2], M. Ensinck[2], A. Ramalho[3], Z. Debyser[1], F. Vermeulen[3], R. Gijsbers[1], M. Carlon[2]

[1]KU Leuven, ADVANTAGE: Advanced Disease Modelling, Targeted Drug Discovery and Gene Therapy, Leuven, Belgium, [2]KU Leuven, Department of Chronic Diseases and Metabolism, Leuven, Belgium, [3]KU Leuven, Department of Development and Regeneration, Leuven, Belgium

Introduction: In the last decade, highly effective CFTR modulator therapy has revolutionized the treatment of cystic fibrosis (CF). However, the most severe CF-causing variants – namely, indel and nonsense mutations – remain completely refractory to modulators, leaving ~10% of the individuals with CF without viable treatment options. A promising therapeutic approach for these challenging mutations is gene editing – which, through a one-time administration, could lead to their targeted and durable correction in the genome of clinically relevant cell types. While several CF-causing nonsense mutations are amenable to base editing, indels can only be corrected by prime editing (PE) owing to the unique capability of the latter to introduce any type of point mutation in the genome. The clinical translation of prime editing would be greatly expedited by a "hit-and-run" delivery system, which could guarantee the desired on-target gene correction while minimizing the risk of off-target edits that is associated with long-term expression of the gene editing machinery. Virus-like particles (VLPs), which transiently deliver the gene editing machinery in the form of a ribonucleoprotein, are ideal candidates for this purpose.
Methods and results: Here, we leveraged PE to tackle W1282X (c.3846G>A) and c.3528del – the fifth and 23rd most common CF-causing *CFTR* variants, respectively. While W1282X could theoretically be corrected though base editing, we found that this strategy resulted in the introduction of deleterious bystander edits.
We tested several prime editing approaches in HEK293T cells expressing the mutant *CFTR*-cDNA, and identified optimal strategies that precisely corrected W1282X and c.3528del with an efficiency of 8 ± 1% and 40 ± 2 %, respectively. The editing restored similar percentages of CFTR ion channel function, which was measured through halide-sensitive YFP quenching. We then validated the best editing strategies in patient-derived intestinal organoids – a highly clinically relevant model. Transduction of the organoids with lentiviral vectors encoding the components of the prime editing machinery resulted in the rescue of CFTR function, which we assessed by measuring forskolin-induced organoid swelling. In parallel, we focused on the improvement of VLPs as delivery vehicles for PE RNPs. Through the refinement of culture and transfection conditions of producer cells, as well as VLP architecture and purification strategies, we were able to achieve a ~10-fold increase in the efficiency of VLP-mediated PE in a fluorescence-based HEK293T reporter system.
Conclusions: Our results showcase prime editing as a uniquely versatile tool that can be harnessed to precisely and permanently correct challenging CF-causing mutations like W1282X and c.3528delC. Efforts to further optimize VLPs with the aim of achieving robust VLP-mediated PE in organoids are currently ongoing.

29 March — 16:30–17:30

Closing Keynote Lecture

Advancing therapies for cystic fibrosis in an evolving global landscape

Deepika Polineni

Washington University in St. Louis (St. Louis, MO, USA)

Cystic fibrosis (CF) is a multiracial and multiethnic genetic disease with significant morbidity and mortality around the globe. While the rise of CFTR modulator therapies has significantly altered the mainstay of CF treatment and projected survival paradigms, still many people with CF continue to wait for diagnostic, therapeutic, and scientific advances to support their health. The changing face of CF is characterized by an evolving demographic and molecular epidemiologic characterization. This remains essential to ensure people with CF receive timely diagnosis and development of CFTR focused treatment strategies that address the diversity of CFTR pathogenic variants. Current efforts around the globe toward clinical recognition and characterization of CF continue to shed light on the heterogeneous needs of the CF population. Importantly, there is an increasing number of clinical trials aimed toward treating people with CF who have CFTR variants that are not responsive to CFTR modulators. However, there are distinct challenges for developing these therapies ranging from addressing fundamental barriers in targeting nucleic acid-based therapies in the CF lung to studying a smaller number of people with CF in these trials, when compared with traditional CF small molecule drug trials. Emerging strategies are informed by clinical trials with an expanding lens on the applications of CFTR modulator therapies, as well as initiatives to address a growing pipeline of nucleic-acid based therapeutic approaches aimed for the smaller population of people with CF who have non-responsive variants for CFTR modulator therapy. This lecture aims to address an evolving landscape of CF highlighting advances, needs, and next directives in bringing novel therapies forward to people with CF.

P1

DNA Methylation of Long Interspersed Nuclear Element 1 (LINE-1) in nasal epithelial cells from Georgian cystic fibrosis patients with rare c.1545_1546delTA pathogenic variant

E. Kvaratskhelia[1,2], S. Surmava[1], M. Gagua[2], E. Maisuradze, L. Livshits[3], K. Kankava[1], E. Abzianidze[1,4], T. Tkemaladze[1,5]

[1]Tbilisi State Medical University, Molecular and Medical Genetics, Tbilisi, Georgia, [2]Tbilisi State Medical University, V. Bakhutashvili Institute of Medical Biotechnology, Tbilisi, Georgia, [3]Institute of Molecular Biology and Genetics of the National Academy of Sciences of Ukraine, Laboratory of Molecular Genetics Human Genomics, Kyiv, Ukraine, [4]Ivane Beritashvili Center Of Experimental Biomedicine, Tbilisi, Georgia, [5]Tbilisi State Medical University, Givi Zhvania Pediatric University Clinic, Tbilisi, Georgia

Introduction: More than 2000 variants of the cystic fibrosis transmembrane conductance regulator (CFTR) gene have been reported worldwide, and cystic fibrosis (CF) is caused by ~300 pathogenic CFTR variants. Molecular genetic analysis of CF patients in Georgia revealed a relatively high frequency of c.1545_1546delTA, a frameshift variant that results in premature termination of protein translation, classified as a class I mutation. Genotype-phenotype correlations in CF twins showed that modifier genes and epigenetic factors also contribute to phenotypic variability in CF patients. Moreover, number of studies have shown that DNA methylation profiling is a valuable biomarker of lung disease severity in cystic fibrosis. In this study we examined methylation levels of Long Interspersed Nuclear Element 1 (LINE-1) in nasal epithelial cells obtained from Georgian CF patients with the c.1545_1546delTA mutation.

Methods: The study was approved by the Ethics Committee of the Tbilisi State Medical University (Tbilisi, Georgia). Nasal epithelial cell (NEC) samples were collected from the inferior turbinate from CF patients (n = 18; age 3.7 ± 2.294) homozygous for the c.1545_1546delTA variant (n=9) or compound heterozygous for the c.1545_1546delTA/ c.1521_1523del (n=10) variants and healthy controls with no history of airway disease (n = 16; age 5.2 ± 6.324). Genomic DNA was extracted from NECs using the QIAamp DNA Mini kit (Qiagen, US). DNA Methylation Analysis of LINE-1 performed using Global DNA Methylation LINE-1 Assay (Active Motif, Carlsbad, CA).

Results: We demonstrated significant loss of methylation in LINE-1 elements in CF patients compared to controls (p=0.0318). Specifically, the methylation levels of LINE-1 elements were decreased by ~28% in CF patients relative to controls. LINE-1 methylation levels were not significantly different between c.1545_1546delTA/ c.1545_1546delTA homozygous and compound heterozygous c.1545_1546delTA/ c.1521_1523del patients (p=0.7351).

Conclusions: Our findings highlight the importance of investigating the mechanisms of epigenetic dysregulation as a valuable biomarker in cystic fibrosis. Further studies are needed to assess the determinants of DNA methylation changes associated with inflammation and lung disease severity in relation to specific mutations.

Acknowledgments: This work was supported by Shota Rustaveli National Science Foundation of Georgia [Grant #FR-22-2601].

P2

Specific stages of iPSC to airway basal cell differentiation exhibit variable organoid swelling in response to CFTR modulators in a cystic fibrosis rare mutation model

X.J. Teoh[1,2], B. Cavanagh[3], A. Berical[4], F. Hawkins[4], D. Kotton[4], P. McNally[5], K. Hurley[1,2], I. Oglesby[1,2]

[1]Royal College of Surgeons in Ireland, Respiratory Medicine, Dublin, Ireland, [2]Royal College of Surgeons in Ireland, Tissue Engineering Research Group, Dublin, Ireland, [3]Royal College of Surgeons in Ireland, School of Pharmacy & Biomolecular Sciences, Dublin, Ireland, [4]Boston University and Boston Medical Center, Center for Regenerative Medicine, Massachusetts, United States, [5] Children's Health Ireland at Crumlin, Dublin, Ireland

Background: The approval of CFTR modulators has brought significant therapeutic benefits and improved the median predicted survival for the majority of people with CF (PwCF). However, a subset of PwCF remain ineligible for these treatments due to their rare, uncharacterised CFTR mutations. The Forskolin-induced organoid swelling (FIS) assay has emerged as a valuable tool for evaluating individual responses to CFTR modulators and predicting clinical efficacy. In this study, we aimed to assess CFTR function in induced pluripotent stem cells (iPSCs) derived from a compound heterozygous individual with two rare mutations c.1493_1507del15/1716G>A. The FIS assay was performed at two specific stages of differentiation to basal-like airway epithelial cell organoids (iBCs): firstly, in early lung progenitor organoids and secondly, in organoids undergoing basal cell induction designated as airway progenitors.

Methods: iPSC lines from a male carrying two rare CFTR mutations and an individual homozygous for the Phe508del mutation (ΔF508/ΔF508) were differentiated towards lung and airway progenitors and iBCs following published protocols. NKX2-1+ lung progenitor cells and iBCs were enriched and purified based on surface markers carboxypeptidase M (CPM), an NKX2-1 surrogate and canonical basal cell marker, nerve growth factor receptor (NGFR) respectively using magnetic activated cell sorting (MACS). The FIS assay was performed on Day 30 lung progenitor cell organoids in 'Airway media' and at Day36+ airway progenitors cultured in 'Basal cell media' with Ivacaftor (IVA) alone or triple-combination therapy consisting of Elexacaftor, Tezacaftor and Ivacaftor (ETI) and 5μM Forskolin. CFTR mRNA was measured by qRT-PCR.

Results: All lines generated high expression of NKX2-1+ lung progenitors (>85%) and NGFR+ iBCs (>90%) post-sort. Day 30 lung progenitor cells derived from F508del iPSCs (known moderate responder to ETI) showed no response to IVA and ~13% swelling in response to ETI. Repeated FIS assays in airway progenitor cells cultured in 'basal cell media' resulted in increased swelling of ~30%. Similarly, the rare mutation line showed no response to IVA in lung progenitor cells and minimal swelling (~3%) with ETI. However, as cells began to adopt a basal cell programme, a notable improvement in response to both IVA and ETI (>25% swelling) was observed indicating enhancement of CFTR function as cells are further matured towards basal-like airway epithelial cells. CFTR mRNA remained unchanged between stages examined however did increase with modulator treatment post-NGFR sort (ETI > IVA).

Conclusion: Efficient and reproducible generation of lung/airway progenitors and airway basal-like cells from CF iPSC lines was demonstrated. Our preliminary data revealed that different cellular stages of the differentiation process exhibit variable responses to CFTR modulators as measured by the FIS assay. As cells transitioned from a basal cell program towards airway basal cells, the major stem cell of the human conducting airway epithelium, response to modulator treatment was enhanced in both the control F508 homozygous moderate responder and the rare genotype. This work builds on previous studies and suggests that personalised CFTR functional assessment in an iPSC-derived airway organoid model should be examined at later differentiation stages than previously described.

P3

Antisense oligonucleotide amenability of splicing and nonsense mutations in cystic fibrosis

M.D Leahy, L.A Clarke , M.D Amaral

BioISI - Biosystems and Integrative Sciences Institute, Faculty of Sciences, University of Lisboa, Lisbon, Portugal

Introduction: Cystic fibrosis (CF) is an autosomal recessive genetic disease characterised by loss of function in the CF transmembrane conductance regulator (CFTR) gene. CFTR modulators (CFTRm) and other therapeutic strategies have been approved for certain CFTR genotypes, however, ~20% of people with CF (pwCF), particularly those with splicing or nonsense mutations, do not qualify for any of these available treatments [1]. Antisense oligonucleotides (ASOs) are synthetic polymers designed to bind to mRNA and modulate gene expression by inducing mRNA degradation, altering pre-mRNA splicing or inhibiting nonsense-mediated decay [2]. ASOs have emerged as a promising strategy for a wide range of genetic diseases. In CF, ASOs could offer the potential for personalised treatments for pwCF who do not respond to CFTRm. Recently, a framework was established to identify rare disease variants that could be corrected using splice-switching ASOs. However, no comprehensive survey has been conducted to evaluate the potential of CFTR variants for splice-switching ASO-based therapies.
Objectives: To apply the existing framework to a comprehensive list of documented CFTR variants. Additionally, we developed a framework to identify nonsense mutations that may be amenable to ASO correction strategies.
Methodology: Data on CFTR variants were obtained from the CFTR2 database (cftr2.org) and the CF Mutation Database (www.genet.sickkids.on.ca/). Predicted amenability to splice-switching ASO therapy was assessed using the taxonomy designed by Kim et al [3]. This assessment involved: (1) Evaluating the predicted impact on canonical splicing using SpliceAI, MaxEntScan and LaBranchoR, alongside damage to coding function by REVEL and (2) Determining the likelihood of ASO-mediated rescue of mis-splicing events, based on the available literature, SpliceAI and MaxEntScan predictions. For nonsense mutations, amenability was evaluated based on their location in the CFTR transcript, their position within exons, and the feasibility of in-frame exon skipping to restore function. Amenability to two key strategies was assessed: (1) Block of downstream exon junction complexes (EJCs) and (2) skipping of nonsense mutation-containing exons.
Results: A total of 1,085 CF-causing variants were identified in the CFTR2 database and analysed. Several splicing and nonsense mutations were identified as likely candidates for ASO correction through steric blocking at or near the mutation site. Additionally, numerous nonsense mutations were identified as potential targets for exon skipping or EJC blocking strategies.
Significance: This study identified several CFTR splicing and nonsense mutations that may be amenable to ASO-based therapeutic strategies and identified a number of potentially correctable variants for which no therapeutic strategies have been developed. These findings lay the groundwork for further investigation into ASO correction strategies and may lead to novel treatments for pwCF with CFTR variants that are currently untreatable.
***Acknowledgements**: Work supported by UID/04046/2025 (to BioISI), UIDB/00100/2020 and UIDP/00100/2020 (to CQE) centre grants from FCT/MCTES, Portugal and research grants SRC 026 from CF Trust (UK) and ORGESTRA Doctoral Network (HORIZON-MSCA-2022-DN-01-01-101120108) from EU.*
[1] Zolin, A., et al. (2024). ECFSPR Annual Report 2022.
[2] Kim, Y. J., & Krainer, A. R. (2023). Mol. Cells, 46(1), 10–20.
[3] Kim, J., et al. (2023). Nature, 619, 828–836.

P4

Novel RNA binding proteins regulate the levels of PTC-containing CFTR mRNA

A. Almeida Henriques, A. Abrantes, C.M Farinha

BioISI - BioSystems & Integrative Sciences Institute, Faculty of Sciences, University of Lisbon, Lisboa, Portugal

The *CFTR* gene encompasses over 2,100 documented genetic variants, grouped into classes (Class I-VII) based on their impact on the CFTR protein. Among these, F508del, a Class II variant, stands out as the most prevalent mutation in persons with cystic fibrosis (pwCF). While modulators are available for the treatment of F508del (and several other variants), approximately 10-15% of pwCF still lack a specific treatment, including those with nonsense mutations.

Nonsense variants (Class I) greatly disrupt protein production, with 178 mutations identified in the CF Mutation Database. These variants introduce premature stop codons (PTCs), leading to the activation of nonsense-mediated decay (NMD), a cellular process that degrades mRNA containing PTCs. Notably, the G542X mutation (c.1624G>T) is the most prevalent among nonsense variants [1]. Customized strategies are crucial, beyond F508del, as understanding CFTR variants is essential for advancing personalized treatments that effectively address the diverse needs of PWCF. Our aim is to understand the network of differentially expressed transcripts and proteins in cells bearing nonsense variants and modulate their expression to find targets to modulate the NMD of the CFTR transcript and circumvent the absence of protein in these cells.

We used transcriptomics and proteomics datasets from 16HBE14o- gene edited cell lines with WT and 6 different CF-causing variants [2] and focused on G542X mutation specific signatures. After validation, using qRT-PCR, we modulated target expression to assess their effect on overall CFTR mRNA expression.

Results: We identified 201 differentially expressed transcripts and 35 differentially expressed proteins unique for G542X. Gene Ontology Enrichment analysis reveals that GO Biological process terms such as “regulation of transcription” and “regulation of RNA metabolic process” are most representative for the transcripts whereas “Protein Biosynthesis” and “RNA-binding” are enriched for proteins – suggesting the association of these datasets with processes affecting the expression of CFTR mRNA in cells bearing G542X-CFTR. Validation of the identified transcripts revealed 7 upregulated and 4 downregulated genes. We are currently knocking down upregulated genes, and performing overexpression of plasmids expressing downregulated genes, assessing their effect in the overall CFTR mRNA and protein levels. Ongoing work identified that the knockdown of the RNA binding proteins TUT1 (Terminal Uridylyl Transferase 1) and HNRNPUL2 (Heterogeneous Nuclear Ribonucleoprotein U Like 2) has a considerable effect in the levels of CFTR mRNA in G542X cells. A similar effect was found under overexpression ZNF793 (Zinc Finger Protein 793) in the same G542X cell line. We are currently evaluating the effect on Protein expression and performing combination experiments with other strategies to identify synergistic and additive effects that can be explored in pwCF that currently lack therapeutic options.

We have identified novel RNA binding proteins as regulators of the abundance of G542X CFTR mRNA. This will allow us to test various strategies to correct nonsense variants and uncover a combined approach where our identified targets lead to NMD inhibition and PTC readthrough to rescue CFTR in these individuals.

Acknowledgements: Work supported by Emily’s Entourage and center grants to BioISI UIDB/04046/2020 and UIDP/04046/2020 (https://doi.org/10.54499/UIDB/04046/2020) from FCT, Portugal (to BioISI)

[1]-CFTR2.[www.cftr2.org]

[2]-[https://doi.org/10.1186/s13578-023-00975-y]

P5

Optimization of blocking oligonucleotides as therapies to correct consequences of deep intronic mutations

C. Felgerolle, K. Deletang, M. Taulan-Cadars

Phymedexp Institute, Inserm U1046, CNRS UMR 9214, Université de Montpellier, Montpellier, France

Introduction: Since years, several CFTR therapeutic strategies have been approved and the number of *CFTR* mutations targeted by therapeutic agents is increasing. Nevertheless, approximately 10%-20% of patients remain not eligible to CFTR modulators, particularly patients with splicing mutations (~12% of the reported *CFTR* mutations). We specifically focused on deep intronic mutations that induce aberrant splicing patterns, mostly the insertion of an intronic region in the mRNA sequence (cryptic exon, CE), generally leading to creation of a premature termination codon. We used an approach of modified oligonucleotides (Target Site Blockers, TSB), to specifically target and block deep intronic mutations effect through their binding on aberrant splicing sites of *CFTR* pre-mRNA. Previously, we designed TSBs for 10 deep intronic mutations and assessed their effectiveness on various cell models. Our promising results lead us to optimize TSB design and delivery.
Materials and methods: TSB optimization means to make transfected tissues generating wider proportion of WT mRNA. This includes the choice of chemical modifications added on ribose constitutive of TSB and of the agents of delivery. Regarding the first aspect, we designed TSBs with different chemical modifications already published and/or used in clinical trials (*Locked Nucleic Acid*, LNA ; *O-Methoxyethyl*, OME ; *O-Methyl*, OM). We also vary proportions of modified nucleotides as well as their positions in TSBs sequence. Thus, for each TSB, this optimization step may generate a lot of new TSBs to test. To help us in screening all these TSBs, we have elaborated new minigenes containing *Firefly Luciferase* gene sequence interrupted by mutated intronic sequences (400-600pb). Thus, Luciferase would be expressed only in normal splicing conditions. For each mutation studied, transfecting bronchial Beas-2B cells with the specific minigene and with a TSB, and then recording Luciferase activity of these cells allowed us to assess the efficacy of TSB to induce normal splicing. Moreover, as Luciferase activity is quantifiable, efficiency of TSBs targeting a same mutation can be compared. This technique allows us a faster optimization of TSB.
Results: Previous assays showed that oligonucleotides were efficient to block inclusion of CE for several mutations, with an increase by 20-30% of normal mRNA rate, up to 70% for c.1680-883A>G and c.1680-886A>G mutations, in bronchial cell lines and in primary nasal cultures. To optimize TSB design, we performed Luciferase minigene assays for mutations c.1680-883A>G, c.1680-886A>G and c.3874-4522A>G. For each mutation, TSBs vary through their nature, number and position of modified nucleotides, but also through their sequences by 1 or 2 nucleotides. For these two mutations, our results show an increased efficiency of LNA-modified TSB while reducing the number of modified nucleotides, even at low concentration (50nM). We are now focusing on agents of delivery to upgrade TSBs cell and nuclear internalization. We also keep optimizing TSBs against other intronic mutations by implementing the Luciferase minigene assay.
Conclusion: Our findings demonstrated the efficacy of TSB-based strategy to restore normal *CFTR* mRNA and we propose optimizing TSB design. TSB-based strategy is an appropriate manner to offer new therapy purposes for CF patients harboring deep intronic mutations.

P6

Nonsense suppression of CFTR PTC variants through attenuation of translation termination and mTOR inhibition

F. Liang, H. Shang, A. Pande , S. Unger , Y. Cheng , S. Cantu , L. Wang , K. Coote , H. Bihler , M. Mense

Cystic Fibrosis Foundation, CFFT Lab, Lexington, United States

Background: Nonsense suppression is a natural biological process where ribosomal translation occasionally occurs at a premature termination codon (PTC) or nonsense mutation in mRNA. Despite significant efforts to develop therapeutic modulators to stimulate PTC readthrough, the biochemical and cellular processes underlying ribosomal readthrough modulation remain largely unknown.
Ribosome-binding antibiotics, such as Geneticin (G418), have been shown to facilitate translational readthrough of PTC mutations in multiple mRNAs, including those responsible for disease-causing CFTR variants. High throughput screening (HTS) aimed at identifying readthrough modulators has revealed chemical modulators with diverse structures, most notably, ribosome-binding antibiotics and degraders of the eucaryotic release factor 1 (eRF1) [1]. Biological profiling of readthrough modulating agents aims to identify targets and better understand the biological mechanisms of translational readthrough. Here, we evaluate the effects of two classes of readthrough modulators on nonsense mutation suppression: eRF modulators and mTOR inhibitors.
Results: Translational readthrough of native CFTR R1162X was observed when CFF 16HBEge CFTR R1162X cells were treated with CC-90009, a degrader of the translation termination factor eRF3a. Combining aminoglycosides (G418, ELX-02) with CC-90009 resulted in the readthrough of native CFTR R1162X, achieving significant levels of full-length CFTR protein equivalent to 20% of the normal CFTR expression in parental 16HBE14o- cells. Additionally, the functional restoration of CFTR R1162X by ELX-02 in the TECC-24 assay was significantly (~3-fold) enhanced in the presence of CC-90009.
HTS of the ReFrame drug repurposing library at the CFFT Lab identified six known, chemical diverse mTOR inhibitors. Two commercially available analogs of one of the mTOR inhibitor hits were also tested and confirmed in the HTS assay. Preliminary results suggest that the hit compounds with mTOR inhibitor activity suppress nonsense-mediated decay (NMD) and ribotoxic stress response (RSR).
Conclusion: Modulator screens have validated ribosome-binding antibiotics and eRF modulators for readthrough and have revealed additional mechanisms. A better understanding of the biological processes of nonsense suppression is crucial for developing therapeutic strategies to manage diseases caused by PTCs. For example, understanding the signaling/mechanisms of mTOR inhibitors may allow the identification of a more specific target for readthrough in CF.
[1] Sharma J et al. A small molecule that induces translational readthrough of CFTR nonsense mutations by eRF1 depletion. Nat Commun. 2021;12:4358. http://doi.org/10.1038/s41467-021-24575-x.

P7

Novel readthrough compounds for the treatment of cystic fibrosis patients with nonsense mutations

I. Pranke[1,2], A. Hatton[1,2], M. Djumagulov[3], E. Dreano[1,2], M. Kelly[1,2], A. Hinzpeter[1,2], L. Bidou[4], O. Namy[4], J.-P. Renaud[3], I. Sermet-Gaudelus[1,2]

[1]Institut Necker Enfants Malades, Paris, France, [2]Université Paris Cité, Paris, France, [3]Urania Therapeutics, Strasbourg, France, [4] Institute for Integrative Biology of the Cell, Gif-sur-Yvette, France

Drugs developed to treat patients with nonsense mutations are not efficient enough to induce readthrough or too toxic to be administered lifelong. A "structure-based drug design" approach on the 80S eukaryotic ribosome lead to new, more potent readthrough compounds, called URN molecules.
Aim: Evaluate the efficacy of novel URN molecules to rescue CFTR with premature termination codon (PTC) in heterologous and native cell models.
A heterologous β-galactosidase/luciferase dual gene reporter system was used to measure the readthrough activity on various PTCs in HeLa cells. 16HBEge cells, primary human nasal epithelial (HNE) cells, and intestinal organoids, carrying G542X or Y122X , were treated with URN molecules and compared to ELX-02 at 100/200 µM for 48h, alone or in combination with a NMD inhibitor (SMG1, 0.5µM/24h) and ETI (VX-661 3µM/VX-445 3µM/VX-770 100nM). CFTR protein activity was assessed by measuring the CFTR-dependent chloride current in Ussing chamber experiments (Isc) or MTECC automated experiments (Ieq). CFTR transcript levels were determined by qPCR.
The URN compounds demonstrated an increased readthrough activity as compared to the reference compound ELX-02 in all tested nonsense mutations including all three stop codons: UGA (G542X, W1282X and S1196X), UAG (W846X) and UAA (Y122X) in the dual reporter system.
In 16HBEge cells, URN-1 and URN-2 (200 µM) demonstrated functional restoration, corresponding to readthrough efficiency, of G542X-CFTR up to 7.5%(±1.5) and 9.4%(±0.6) of the wild type (WT) activity, respectively. This was at significantly higher level than ELX-02 increasing CFTR function only up to 1.2% of WT.
In G542X-CFTR HNE cells, URN1 at 200µM rescued CFTR activity up to 4.5%(±0.2) of the WT as compared to ELX-02 (200 µM) (0.3%(±0.1)). URN-2 presented higher efficiency at 100 µM, correcting G542X-CFTR in HNE cells up to 6%(±0.5) of the WT. As compared, ELX-02 at 100 µM in the same cells was less efficient, inducing CFTR function up to 0.2%(±0.1) of the WT. URN-2 (100 µM) was also significantly more efficient than ELX-02 (100 µM) for Y122X in HNE cells, resulting in correction up to 8% of WT-CFTR, as compared to ELX-02 (5%).
In 2D intestinal organoids, URN-1 (200 µM) significantly corrected G542X-CFTR up to 8.3%(±3) of WT-CFTR. URN-2 presented higher efficiency, because improved G542X-CFTR up to 7.8%(±0.9) of the WT at only 100 µM concentration. ELX-02 (100µM) corrected CFTR activity up to only 0.5%(±0.1). URN-02 at 100 µM in organoids from Y122X-homozygous patients presented efficiency of correction up to 15%(±0.8) of the WT-CFTR activity, which was higher than correction obtained upon ELX-02 100 µM reaching 13%(±1.9) of the WT.
CFTR function was further increased by addition of the corrector VX-661/445 and NMDi on top of URN-1/URN-2. Improvement of CFTR function was associated with increase in the level of CFTR transcripts providing evidence of additional NMD inhibition upon URN compounds.

A structure-based drug design approach yielded compounds that efficiently readthrough CFTR UGA and UAA PTCs. URN molecules are more efficient than ELX-02 to rescue G542X- and Y122X-CFTR activity in heterologous and primary in vitro models.

P8

Developing novel ribosome-directed small molecules and antisense oligonucleotides as therapeutic approaches for refractory CFTR variants

J.J. Jackson [1,2], C. Foye[1,2], A.G. Winters [1,2], E. Freestone[1,2], D. Patel[1,3], Y. Du[4,5], L. Huang[6], K.E. Oliver[1,2]

[1]Department of Pediatrics, Emory University School of Medicine, Atlanta, United States, [2]Center for Cystic Fibrosis and Airways Disease Research, Children's Healthcare of Atlanta and Emory University School of Medicine, Atlanta, United States, [3]Center for ViroScience and Cure, Laboratory of Biochemical Pharmacology, Children's Healthcare of Atlanta and Emory University School of Medicine, Atlanta, United States, [4]Department of Pharmacology and Chemical Biology, Emory University School of Medicine, Atlanta, United States, [5]Emory Chemical Biology Discovery Center, Emory University School of Medicine, Atlanta, United States, [6]Ionis Pharmaceuticals, Carlsbad, United States

Background: Of the 2,121 *CFTR* variants reported to date, 271 are on-label for clinically approved CFTR modulators in the United States. *CFTR* variants that have demonstrated insensitivity to these compounds, and/or have not been tested for drug responsiveness, largely encompass severe defects in CFTR synthesis (e.g. Class I variants) and processing (e.g. Class II variants). Among Class I variants, nonsense alleles are particularly difficult to overcome due to premature degradation of the encoded mRNA and protein. Our work endeavors to identify and therapeutically target genetic interactions that influence biogenesis of these and other refractory *CFTR* variants. We previously discovered ribosomal protein L12 (RPL12/uL11) as a robust modifier of mutant CFTR processing, with ~50% knockdown of RPL12 conferring improved functional expression of specific variants from different *CFTR* subclasses (e.g. F508del, W1282X). In the present study, novel antisense oligonucleotides (ASOs) and small molecule inhibitors of RPL12 were developed and evaluated for potential to rescue the same *CFTR* variants.

Methods: ASOs targeting human RPL12 were engineered by Ionis Pharmaceuticals. These molecules were tested for efficacy in CF bronchial epithelia (CFBE41o-) and primary human nasal epithelia (HNE) encoding wild-type or F508del-CFTR. For high-throughput drug screening (HTS), Fischer rat thyroid (FRT) cells were stably transduced with human RPL12 or W1282X-CFTR encoding a C-terminal, in-frame NanoLuc reporter. Measures of cell viability (Promega CellTiter-Fluor Assay), RPL12 or CFTR luciferase signal (Promega NanoGlo Assay), CFTR mRNA expression (qRT-PCR), protein maturation (western blot), and/or channel function (Ussing chamber) were employed. Studies included treatment comparisons to negative controls (scrambled ASO, empty vehicle) and positive controls such as established inhibitors of translation (G418, PTC-124, ELX-02, Escin).

Results: In CFBE and HNE, we show two ASOs decrease RPL12 transcripts and protein to similar levels achieved with siRNAs. These RPL12 ASOs significantly augment wild-type and F508del-CFTR band C maturation, in addition to F508del-dependent short-circuit currents. In FRT cells, early HTS results revealed 47 compounds at which ~50% suppression of RPL12 is attained. These hits are presently undergoing: (1) dose-response confirmatory screens; (2) counter-screens with cell viability to exclude compounds demonstrating toxicity; (3) counter-screens against NanoLuc protein alone as a measure of specificity; (4) application to FRT cells expressing W1282X::NanoLuc to evaluate readthrough efficiency; and (5) structure-activity relationship assessments to optimize functional group modifications that correlate with improved RPL12 suppression and mutant CFTR rescue.

Conclusions: Partial depletion of RPL12 levels represents a feasible strategy for CFTR modulation, which may be applicable to *CFTR* genotypes refractory to available clinical interventions. Our work serves as a foundation from which future investigations may be pursued to examine efficacy and tolerability of anti-RPL12 compounds or ASOs delivered to CF animal models. This study was supported by the NIH, U.S. CFF, and Atlanta Pediatric Research Alliance.

P9

Inflammatory stimuli enhance pharmacological rescue of CFTR nonsense mutations

A. Borrelli[1], A. Venturini[1], D. Guidone[1], M. De Santis[1], F. Ciciriello [2], L.J.V. Galletta[1]

[1]TIGEM- Telethon Institute of Genetics and Medicine, Cell Biology, Pozzuoli, Italy, [2]Bambino Gesù Children's Hospital, IRCCS, Pneumology and cystic fibrosis unit , Rome, Italy

Background: Nonsense mutations in the coding sequence of the *CFTR* gene cause premature termination codons (PTCs) that hamper the normal CFTR protein translation. They result in severely truncated forms of the CFTR chloride channel that are not sensitive to present CFTR modulators. Pharmacological stimulation of read-through (RT) ability of the ribosome stalled at the PTC site can overcome abnormal truncation, allowing continuation of the protein synthesis. Production of a full-length CFTR protein can be achieved by ELX-02, a compound currently under clinical investigation as a RT agent suitable for people with cystic fibrosis (pwCF). Recently, small molecules acting as eRF3a degraders, such as CC-90009, have been described as effective on PTCs (1). We evaluated the effect of CC-90009 as a RT agent in combination with ELX-02 on CFTR carrying PTCs. It has been reported that the inflammatory cytokines TNFα and IL-17A enhance the efficacy of CFTR modulators in rescuing F508del-CFTR in HBECs (2). We asked if inflammation could also influence correction of PTCs.
Methods: Bronchial epithelial cells homozygous for the G542X mutations were used to generate differentiated epithelia on porous supports under air-liquid interface (ALI) condition. Epithelia were treated (72 h) with/without IL-4 or TNFα/IL-17A. Cells were also treated in the last 24 h with ELX-02 (200 μM) plus VX-809 (1 μM), plus/minus CC-90009 (0.1 μM). We evaluated the rescue of CFTR function elicited by the different drug combinations and cytokine treatments by short-circuit current (Isc) recordings. Automated capillary immunoblot experiments and qRT-PCR were used to detect corrected CFTR protein and mRNA levels.
Results: We found a significant CFTR rescue (three-fold increase of CFTR-dependent current) by CC-90009 in combination with ELX-02 plus VX-809. Importantly, the effect of this triple compound combination was enhanced in cytokine-treated epithelia, with a 15-fold and 9-fold increase in CFTR current elicited by IL-4 and TNFα/IL-17A, respectively. The large rescue of CFTR function was paralleled by the appearance of full-length CFTR protein and by the increase in CFTR mRNA. The effect of inflammatory stimuli on G542X-CFTR could be mediated by enhanced translational RT and/or by inhibition of the nonsense-mediated RNA decay.
Conclusions: We found that cytokine-treated epithelia showed an enhanced response to the triple compound combination containing the eRF3a degrader CC-90009. We are currently investigating the molecular basis of this behavior since the cytokine treatment may change the expression of genes controlling protein synthesis thus potentiating the effect of RT maneuvers.
Our findings may lead to the identification of novel targets to correct the effect of nonsense mutations in CF and other genetic diseases. Our results suggest that pharmacological rescue of CFTR with nonsense mutations could be more effective than expected in vivo due to inflammatory conditions.

1. Lee et al., *J Clin Invest* 132:e154571, 2022
2. Tayyab Rehman et al., *J Clin invest* 131:e150398, 2022

This work was supported by grants from the Cystic Fibrosis Foundation (GALIET22I0), the Italian Cystic Fibrosis Foundation (FFC#9/2022 and GMRF#1/2024), and the Italian Ministry of Health (GR-2018-12367126).

P10

Identification of novel pharmacological modulators of the NMD mechanism to recover CFTR function in patients with nonsense mutations

A. Venturini[1], A. Borrelli[1], F. Ciciriello[2], L.J. Galietta[1]

[1]TIGEM - Telethon Institute of Genetics and Medicine, Pozzuoli, Italy, [2]Cystic Fibrosis Center, "Bambino Gesù" Children's Hospital, IRCCS, Rome, Italy

Background: Nearly 10% of CFTR mutations, particularly premature termination codons (PTCs), are insensitive to CFTR modulators (i.e. correctors and potentiators). The pharmacological rescue of PTCs requires a combinatorial approach. First, compounds that inhibit the nonsense-mediated RNA decay (NMD) mechanism are crucial to increase the amount of CFTR transcripts. In addition, readthrough agents are required to allow the continuation of protein synthesis, by inserting an amino acid in the site of PTC. NMD inhibitors can also be effective in the absence of a readthrough agent for PTCs localized at the carboxy-terminus of the CFTR sequence such as W1282X. The aim of this project was to identify novel molecules able to modulate the NMD mechanism.
Methods: We used the 16HBE14o- cell line expressing W1282X-CFTR (obtained from CFF) and stably transfected with the halide-sensitive yellow fluorescent protein (HS-YFP) to screen a chemical library of more than 9,000 compounds, including drugs, investigational drugs, and compounds with known biological activity (mechanistic probes). The most effective compounds have then been characterized by secondary biochemical and functional assays to understand their mechanism of action.
Results: We have completed the screening by the HS-YFP functional assay, and we identified 75 hits. Molecules showing the greater effect on CFTR rescue were further tested in the presence/absence of CFTRinh-172 to confirm a direct activity on CFTR channel and not on other anion channels/transporters. We focused our attention on three different compounds: NMDi-01, NMDi-02, NMDi-03. We evaluated their effects both at the transcriptional and protein expression level. These compounds induced more than 10-fold increase in CFTR-mRNA transcripts and promoted the appearance of a signal for CFTR protein. We then tested their efficacy on W1282X mutation at the functional level, by conducting short-circuit current recordings experiments. NMDi-01 and NMDi-02, in combination with CFTR correctors, were able to induce a marked increase in CFTR channel activity. Interestingly, NMDi-03 *per se* elicited a significant increase in CFTR function, that was further amplified when combined with correctors. To obtain further information about the mechanism of action of active compounds, we performed an analysis of their effect on 16HBE14o- cell transcriptome, both the wild type and W1282X versions. Preliminary results show that CFTR transcript is specifically upregulated in W1282X cells, in agreement with NMD specifically affecting the CFTR mRNA with PTC. A deep bioinformatic analysis is being carried out to analyze the data obtained by RNA seq. The goal is to highlight common and compound-specific signatures elicited by treatments.
Conclusions: We have identified three possible candidates acting as NMD inhibitors/modulators that could increase CFTR rescue in patients with PTCs.

Acknowledgements: This project is supported by CFF (GALIET22I0), the European Cystic Fibrosis Society and CF Europe (post-doctoral research fellowship to Arianna Venturini).

P11

The cereblon E3 ligase modulator, CC-90009, rescue CFTR nonsense variants promoting NMD suppression and PTCs readthrough in rectal organoids

R.V. Latorre[1], C. Mortali[1], D. Scutelnic[2], M.M. Bogdan[3], K. Kleinfelder[1], M.M. Mina[1], A. Massella[4], F. Tomba[4], C. Daffara[2], P. Melotti[5], C. Sorio[1]

[1]University of Verona, Department of Medicine, Verona, Italy, [2]University of Verona, Department of Computer Science, Verona, Italy , [3]University of Verona, Department of Engineering for Innovation Medicine, Verona, Italy, [4]Azienda Ospedaliera Universitaria Verona, Endoscopy Surgery Unit, Verona, Italy, [5]Azienda Ospedaliera Universitaria Verona, Cystic Fibrosis Centre, Verona, Italy

Background: Approximately 10% of the worldwide cystic fibrosis (CF) population has premature termination codon (PTC) variants, that prevent translation of cystic fibrosis transmembrane conductance regulator (CFTR) full-length protein and trigger nonsense-mediated mRNA decay (NMD). CFTR modulators approval opened a new era for CF precision medicine, but to date no therapies are still available to restore PTC variants. Readthrough agents that target distinct components of the translation machinery represent a promising and feasible treatment strategy. But a comprehensive analysis of a series of primary cells representative of different variants and patients is necessary to evaluate and compare their effectiveness for clinical evaluation.
Aims: Here we investigate the efficacy of the cereblon (CRBN) E3 ubiquitin ligase modulator, CC-90009, in restoring CFTR function and mRNA expression using patient-derived rectal organoids homozygous or compound heterozygous for different PTC variants: R1162X/R1162X (2X), W1282X/W1282X (2X), G542X/G542X, R553X/R1158X, G542X/S466X, R1162X/Q39X.
Methods: Drugs testing were performed on 3D cell model by Forskolin Induced Swelling (FIS) assay and analysed with an innovative AI-based software (SOFTD), designed to simplify the segmentation of organoids used for functional drug testing. CFTR mRNA expression was characterized in response to all compounds by quantitative PCR.
Results: Our data show that CC-90009, in combination or not with CFTR modulators, induces significant increase of FIS rates compared to vehicle in almost all organoid lines. We also observed that the mRNA level is incremented by CC-90009 treatment and significantly correlates with functional recovery, confirming the ability of this eRF3a degrader to suppress the NMD complex and generate a functional protein. Furthermore, the combination with the aminoglycosides G418 or ELX-02 but not with PTC124 further enhances their mutual effects, dramatically increasing the PTC readthrough response.
Conclusions: Our results support the role of eRF3a degrader, CC-90009, alone or in combination with other compounds as a promising therapeutic approach to rescue CFTR nonsense mutations. Additional candidate readthrough molecules are currently under study using our intestinal organoid-based screening and validation platform.

P12

Peptide-based CFTR stabilizers as novel therapeutic tools in cystic fibrosis

C. TEKO-AGBO, M. SINANE, K. DELETANG, C. FELGEROLLE, M. TAULAN-CADARS, P. BOISGUERIN

PHYMEDEXP, INSERM U1046, MONTPELLIER, France

Background: The development of therapies for CF patients has been greatly advanced by modulators comprising "correctors" and "potentiators." However, the current combination of modulators, including Trikafta therapy, is costly and not efficient for all patients, highlighting the need for novel strategies.
In this context, we have reported that an interfering peptide iCAL36 could rescue CFTR activity (11% increase in chloride efflux) (Vouilleme et al 2010, Cushing et al 2010). Subsequent to this, we demonstrated that the vectorization of iCAL36 by a cell-penetrating peptide enhanced cellular internalization, thereby reducing the required peptide concentration while maintaining CFTR accumulation at the apical membrane (Seisel et al 2021). Then, to further optimize the peptide sequence in terms of metabolic stability, we have substituted several positions within the iCAL36 sequence by non-natural amino acid to avoid degradation by proteases.
Objectives: Our project's primary objective is to advance the development of peptide-based CFTR stabilizers (CPP-iPep - sequence confidential), either alone or in combination with current CF modulators to reduce their doses. We will also evaluate if a combination with oligonucleotide-based CFTR stabilizer (CFTR-TSB) could further improve CFTR activity.
Methods: In order to achieve these objectives, peptide-based CFTR stabilizers are first tested in human bronchial epithelial cells 16HBEge-p.Phe508del in terms of internalization(confocal microscopy) and CFTR quantification (Western blot). Then, CFTR channel function using Ussing chamber was also assessed.
Results: We previously demonstrated that the vectorized iCAL peptide (TRI-iCAL36, 10 µM) could increase the cellular CFTR amount only if the 16HBEge-p.Phe508del cells are pre-incubated with VX405/VX809. In this study, we could show for the first time that CPP-iPep could increase cellular CFTR amount without VX pre-incubation in the same way as previously TRI-iCAL36. The good activity could be explained by an important cellular internalization property of the optimized CPP-. This suggests that the optimized peptide CFTR stabilizer could be used with a lower VX concentration. Additionally, our results demonstrated that CPP-iPep (10 µM, 3 h incubation apical) could enhance CFTR activity in a 3D CF primary human bronchial epithelium model cultured in Air Liquid Interface (ALI) without the need of VX405/VX809 addition. Finally, we also confirm that a pre-incubation with CFTR-TSB (50 nM, 24 h incubation apical) prior to the CPP-iPep incubation could further increase CFTR activity.
Conclusion: These findings highlight the potential of peptide-based CFTR stabilizers as an innovative therapy for patients with CF.

P13

CFTR stabilizers as therapeutic tools in cystic fibrosis

M. Sinane, K.Deletang, C. Felgerolle, C. Teko-Agbo, P. Boisguerin, M. Taulan-Cadars

Phymedexp Laboratory – INSERM U1046, Montpellier, Montpellier, France

Scientific Background: The treatment of cystic fibrosis patients has evolved considerably with the development of CFTR channel modulators that increase the amount of CFTR protein addressed to the apical membrane ("correctors") and the probability of CFTR channel opening ("potentiators"). However, the current treatment proposed for CF patients, which is based on the TriKafta tri-therapy can be limited for some patients, has side effects and a high cost. It remains therefore necessary to propose new alternatives therapeutic approaches that could be proposed alone or in combination with the current treatment. In this context, we have developed oligonucleotide- based CFTR stabilizers (named TSB-CFTR, that target CFTR mRNA 3'UTR extremity) which aim to increase CFTR mRNA and protein expression at the apical membrane to moderate the CF phenotype.
Objectives: Our project aims first to push forward the development of the CFTR stabilizers (TSB-CFTR) alone as well as in combination with current CF-modulators. We also performed the first de-risking trials of the stabilizing CFTR (biodistribution and route of administration).
Methods: In order to achieve these objectives, TSB-CFTR are tested in a 3D CF primary human nasal and bronchial epithelium model cultured in Air Liquid Interface (ALI). CFTR stabilizers are incubated in CF cultures and then CFTR mRNA (qPCR) and protein (western blot) amounts are measured, as well as CFTR channel function (Ussing chamber). TSB-CFTR efficacy will be evaluated not only in epithelial cells from CF patients bearing the p.Phe508del mutation (the most frequent mutation in CF) but also other genotypes. To initiate preclinical studies, *in vivo* experiments of TSB-CFTR biodistribution have been carried out. Fluorescent-labelled-TSB-CFTR have been administrated in mice, and fluorescence rate has been measured in several organs (spleen, kidneys, lung…).
Results: We previously demonstrated that the use of TSB-CFTR targeting the 3'UTR extremity of the CFTR mRNA led to increased CFTR mRNA level (Viart V *et al*. 2015) and we first optimize TSB efficacy by testing different chemical modifications. Recent results showed that apical TSB-CFTR application (24h-50nM) induced a marked increase in CFTR mRNA and protein amounts, as well as an improvement in CFTR chloride activity in cells cultured from CF patients. Additionally results revealed that oligonucleotide- based CFTR stabilizers vectorization using Peptide-Based Nanoparticles could further increase the beneficial effect of CFTR-TSB. Finally, first biodistribution assay consisting of an administration of different concentrations of fluorescent CFTR-TSB by nasal inhalation in mice, compared to IP administration, revealed a local distribution of these molecules in lungs.
Conclusions: Our therapeutic approaches are applicable for some mutations not eligible for CF modulators so far, and can be used alone or in combination with existing CF-modulators.

P14

Partial inhibition of proteins in distinct structural regions of the ribosome confer differential rescue of N- versus C-terminal CFTR nonsense variants

A.G. Winters[1,2], E. Freestone[1,2], W. Wang[3], M.H. Ali[1,2], J.J. Jackson[1,2], C. Foye[1,2], J.L. Hartman IV[3,4], E.J. Sorscher[1,2], K.E. Oliver[1,2]

[1]Emory University School of Medicine, Pediatrics, Atlanta, United States, [2]Children's Healthcare of Atlanta and Emory University School of Medicine, Center for Cystic Fibrosis and Airways Disease Research, Atlanta, United States, [3]University of Alabama at Birmingham, Gregory Fleming James Cystic Fibrosis Research Center, Birmingham, United States, [4]University of Alabama at Birmingham, Genetics, Birmingham, United States

Background: Among the U.S. CF population, ~13% of patients encode a premature termination codon (PTC) in *CFTR*, the most prevalent of which are G542X and W1282X. These variants confer severe disease phenotypes and remain without an adequate therapeutic intervention. To address unmet medical needs, our work focuses on elucidating mechanistic impact and therapeutic potential of genetic modifiers that influence PTC biogenesis. In previous studies, we modeled CF in a homologous yeast phenomic system to ascertain gene-gene interaction networks responsible for read-through of CF-causing PTCs. High-throughput analysis revealed several gene deletions, such as ribosomal proteins L12 (RPL12/uL11) and L8 (RPL8/uL2), that significantly improved PTC functional expression.
Methods: For the present study, differential effects of RPL12 or RPL8 depletion conferred to CFTR PTCs were determined with siRNA knockdown using Fischer rat thyroid (FRT) cells, CF bronchial epithelia (CFBE41o$^-$), and primary human nasal epithelia (HNE). CFBE41o$^-$ and FRT were stably transduced with G542X- or W1282X-CFTR cDNA. Additional isogenic FRT lines were engineered to encode an in-frame C-terminal NanoLuc reporter to measure PTC read-through, or a horseradish peroxidase (HRP) tag to detect CFTR cell surface localization. Cell viability, CFTR expression, protein processing, and channel function were monitored. Non-specific siRNA and empty vehicles served as negative controls. G418 (read-through agent) served as a positive control. Tests of additivity/synergy were conducted with elexacaftor-tezacaftor-ivacaftor (ETI).
Results: Silencing RPL12 or RPL8 expression by ~50% enhanced G542X plasma membrane density (~3-fold), although G418 rescue was substantially higher (~17-fold). When RPL12 suppression was combined with ETI, G542X trafficking was augmented ~28-fold. Following depletion of RPL12 or RPL8, cell surface localization of W1282X was increased to the same magnitude achieved by G418 (~6-fold). W1282X plasma membrane density was also improved by ETI application (~15-fold), which was further augmented by silencing RPL8 (~29-fold) or RPL12 (~24-fold). RPL12 suppression enhanced W1282X::NanoLuc signals (~3-fold) and full-length band C production for both G542X and W1282X. In addition, W1282X baseline open channel probability was increased by inhibiting RPL8 (~7-fold) or RPL12 (~3-fold). Knockdown of either ribosomal protein significantly augmented W1282X short-circuit currents in FRT, CFBE41o$^-$, and primary HNE (*CFTR*$^{W1282X/W1282X}$ genotype).
Conclusions: RPL12 is a constituent of the GTPase-associated center and resides near the A-site of the peptidyltransferase center, thus serving as a well-known interface for aminoacylated-tRNAs and GTP-bound translation factors. Our previous work shows RPL12 silencing moderately attenuates translation initiation and elongation rates, thereby promoting improved CFTR folding dynamics. Findings from the present study suggest that RPL12 depletion also modestly impairs ribosome fidelity to elicit PTC read-through. In contrast, RPL8 interfaces with the E-site of the peptidyltransferase center and confers subtle structural rearrangements to functional centers of the 60S and 40S subunits. By disrupting these interactions, translational fidelity is likely reduced and therefore enhances PTC read-through. Our collective results indicate partial inhibition of RPL8 or RPL12 should be considered as a novel therapeutic strategy for nonsense suppression, the effects of which may be synergistically augmented by combination with CFTR modulators. This work was supported by the U.S. NIH and CFF.

P15

Searching for Small Molecule Modulators of Premature Termination Codon Variants in the CFTR Gene

L. Clarke[1], M. Leahy[1], H. Botelho[1], V. Cachatra[2], C. Moiteiro[2], M. Amaral[1]

[1]University of Lisboa, Faculty of Sciences, BioISI-Biosystems and Integrative Sciences Institute, Lisboa, Portugal , [2]Centro de Química Estrutural, Institute of Molecular Sciences, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal

Background: Premature termination codons (PTCs) are introduced into CFTR mRNA by nonsense variants, which account for a significant proportion of total known CFTR variants (8.4%), and for which there are still no approved CFTR modulator (CFTRm) therapies. The effect of such variants is generally two-fold: *i)* the presence of the PTC triggers mRNA transcript degradation by the nonsense-mediated decay (NMD) mechanism [1]; and *ii)* any resulting translated protein is truncated and thus, almost always, non-functional. Such variants are therefore associated with severe CF phenotypes, and there is thus an urgent need to find novel therapeutic strategies for individuals with CF with PTC mutations, which could include both PTC read-through agents and NMD inhibitors.
Objective: Our aim was to validate novel small molecules to correct the defective processing of CFTR transcripts bearing PTCs, previously identified by screening a small library of unique novel compounds.
Methods: We used a microscopy assay performed in HEK Flp-in cells stably expressing a triple-tagged CFTR PTC mini-gene construct (mCherry G542X-Flag-CFTR-eGFP cDNA with 3 introns between exons 14-17), including mCherry (red) at the N-terminus, eGFP (green) at the C-terminus, and a Flag-tag for detection of CFTR plasma membrane (PM) localization by immunofluorescence with anti-Flag antibody in unpermeabilized cells. In this assay, the readout for NMD suppression is increased red fluorescence from a basal level, and the PTC read-through readout is increased green fluorescence. Two series of novel compounds (a total of 50 compounds) [2, 3] were tested alongside the SMG1i NMD inhibitor and the G418 aminoglycoside readthrough compound as experimental positive controls. Furthermore, compound autofluorescence was determined.
Results: Of the 50 novel compounds screened, we chose the four most promising (compounds 11, 12, 17, and 40) for further investigation. Toxicity was assessed using the Resazurin assay, and found to be negligible at most experimental concentrations, for three of the four compounds. We also measured the effects of varying concentrations of the four compounds on stabilization of CFTR mRNA in gene edited 16-HBE cells expressing three different PTC variants. Three of the four compounds produced significantly enhanced CFTR mRNA abundance at certain concentrations, with some variant specificity, with C11 demonstrating significant effects for G542X/W1282X, C12 for Y122X/W1282X, and C17 for Y122X only. Intestinal organoids with PTC variants are also being used to validate the potential application of these novel compounds in the context of personalized medicine.
Acknowledgements: *Work supported by UID/04046/2025 (to BioISI), UIDB/00100/2020 and UIDP/00100/2020 (to CQE) center grants from FCT/MCTES, Portugal and research grants SRC 026 from CF Trust (UK) and ORGESTRA Doctoral Network (HORIZON-MSCA-2022-DN-01-01-101120108) from EU.*
References: [1] Clarke LA *et al* (2019) *Hum Mutat* **40**: 326-334; [2] Vieira P *et al* (2020) *Chem Eur J* **26**: 888-899; [3] Moiteiro C *et al* (2022) *Eur J Org Chem* **3**: 58-72.

P16

Investigating the therapeutic effects of K+ channel modulators on mucus and airway surface liquid in cystic fibrosis

O. Hamed[1,2], Z. Chen[2], D.C.H. Benton[2], V. Dua[1], G.W. Moss

[1]University College London, Department of Chemical Engineering, London, United Kingdom, [2]University College London, Department of Neuroscience, Physiology and Pharmacology, London, United Kingdom

Epithelial ion transport regulates the depth of airway surface liquid (ASL), a thin fluid layer (0.1-10 μm) lining the airway epithelium. The ASL enables vital mucociliary clearance (MCC) of mucus-trapped pathogens in the upper airways via co-ordinated beating of thousands of cilia. In cystic fibrosis (CF), loss-of-function mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) cause a loss of anion secretion into the ASL, dehydrating the ASL, thus generating sticky mucus that is difficult to clear, resulting in chronic lung infections.

In recent years, CFTR correctors and potentiators have significantly improved quality of life in CF patients. However, these do not fully restore CFTR function, while patients with specific mutations are excluded from treatment. K^+ channels have been demonstrated to play an essential role in ASL regulation by setting the electrochemical driving force for anion secretion. Hence, K^+ channel modulation presents a promising therapeutic strategy for mucus rehydration in CF.

To investigate the potential benefits of K^+ channel modulation, we cultured human bronchial epithelia from CF donors with R347P/ΔF508 and ΔF508/ΔF508 mutations, treating them for 24 hours with ETI (3 μM elexacaftor, 3 μM tezacaftor, 0.5 μM ivacaftor) or ETI with a K^+ channel modulator (KM). The K^+ channel modulator tested has previously passed Phase I and Phase II clinical trials for other conditions.

To quantify the effectiveness of therapeutic target modulation in CF airway models, we used nanosensor probes mounted on a scanning ion conductance microscope (SICM) to measure key airway epithelial characteristics associated with airway disease pathology. This included mucus rheology, cilia coordination and ASL depth. Reduced ciliary co-ordination is increasingly reported as occurring in CF, contributing to impaired MCC, along with mucus rheology. Hence, we also attempted to assess whether treatment could improve ciliary co-ordination by measuring cilia beat frequency (CBF) and assessing a) CBF variance across cultures, b) average variance between all possible pairs of CBF positions within cultures, normalised by distance between the points (twin-point-median-variance, TPMV).

We measured the ASL properties of 7 ETI treated cultures (3 R347P/ΔF508 and 4 ΔF508 homozygotes) in the SICM and then treated them with ETI+KM to be examined the next day. In all 3 cultures where CBF could be measured, there was a mutation-independent, statistically significant ($p<0.05$) reduction in CBF variance and TPMV, indicating improved co-ordination of cilia in the cultures. In all 7 cultures, the variance of the spinnability decreased after ETI+KM-treatment ($p=0.008$), an effect previously reported by us with GoSlo SR-5-6, a potent activator of large-conductance calcium-activated K^+ channels. This effect was replicated when we treated CF cultures with ETI and is in line with the theory that reduced MCC is associated with wider variance in spinnability, perhaps due to the formation of mucus 'clumps' and reflecting reduced homogeneity of CF mucus.

P17

An Investigation of The Importance of pH in Controlling Mucin Rheology

S. Li , D.C.H. Benton, G.W.J. Moss

University College London, London, United Kingdom

We are investigating the importance of pH in controlling mucin rheology. Airway surface liquid (ASL) mucins act as a host defense to trap and remove inhaled pathogens. Altered rheological properties can lead to impaired mucociliary clearance. The ASL is reported to be more viscous in cystic fibrosis (CF), and possibly more acidic, due to defective anion secretion by the cystic fibrosis transmembrane conductance regulator (CFTR). In addition, CO_2 variation in the airways during tidal breathing causes ASL pH oscillations that are thought to be dampened by mucins. An interaction between H^+ and mucins suggests that mucin rheology may change as the CO_2 levels vary. We have therefore measured mucin spinnability using a humidified CO_2/air mixture in a chamber controlled by a built-in CO_2 sensor and equipped with a scanning ion conductance microscope (SICM). The chamber CO_2 concentration can be changed between 0.04% and 5% resembling the changes that occur during respiration. Harvested mucus spinnability was measured as the point at which a mucin thread breaks as it is pulled by the SICM probe during withdrawal. We also measured the pH-dependence of spinnability for crosslinked porcine gastric mucins reconstituted at different pHs. For pig gut mucins the rheology clearly changed, depending on the pH of the buffer in which it was reconstituted. The same correlation was not observed for the spinnability ASL harvested from human bronchial CF airway epithelial cultures suggesting key mucin differences.

P18

The apical mucus layer alters the pharmacological properties of the airway epithelium

D. Guidone[1], M. De Santis[1], E. Pesce[2], V. Capurro[2], N. Pedemonte[2], L.J. Galietta[1,3]

[1]Telethon Institute of Genetics and Medicine, Pozzuoli (Naples), Italy , [2]U.O.C. Genetica Medica, Istituto Giannina Gaslini, Genova, Italy , [3]Department of Translational Medical Sciences, University of Napoli "Federico II", Naples, Italy

Human airway epithelial cells, differentiated in vitro under air-liquid interface condition, represent an efficient model to study electrogenic transepithelial ion transport. In particular, the short-circuit current technique is frequently used to evaluate the extent of CFTR activity, the cAMP-activated chloride channel that is defective in cystic fibrosis (CF). Typically, CFTR activity is estimated from the drop in current caused by $CFTR_{inh}$-172, a selective CFTR inhibitor. Unexpectedly, we found that $CFTR_{inh}$-172, as well as PPQ102, another CFTR inhibitor, only caused partial inhibition of the cAMP-activated current. The effect of inhibitors was further reduced in epithelia treated with pro-inflammatory cytokines (IL4; IL17+TNF-alpha), which are characterized by abundant mucus secretion. The partial effect of inhibitors could suggest that other chloride channels contribute, in addition to CFTR, to the cAMP-activated current. However, we formulated the alternative hypothesis that the mucus layer is responsible for the poor activity of CFTR inhibitors. Accordingly, we treated the epithelial surface with the reducing agent dithiothreitol to remove mucus filaments and bundles. Removal of mucus, confirmed by immunofluorescence, resulted in highly enhanced sensitivity of CFTR to pharmacological inhibition. Our results show that the mucus layer represents an important barrier whose presence limits the activity of pharmacological agents. Therefore, apical washing with a reducing agent appears as a needed step in the preparation of epithelia for short-circuit experiments. This is particularly relevant in studies on CF epithelia evaluating the efficacy of pharmacological interventions to rescue CFTR function.

This work was supported by grants from the Cystic Fibrosis Foundation (GALIET22I0), the Italian Cystic Fibrosis Foundation (FFC#9/2022 and GMRF#1/2024), and the Italian Ministry of Health (GR-2018-12367126).

P19

Ex vivo pig lung as a new Cystic Fibrosis model for the study of *Pseudomonas aeruginosa* biofilm infection and phage therapy application

M. Cafora[1], F. Forti[2], V. Capurro[3], F. Harrison[4], F. Briani[2], A. Pistocchi[1]

[1]University of Milan, Department of Medical Biotechnology and Translational Medicine, Milan, Italy, [2]University of Milan, Department of Biosciences, Milan, Italy, [3]IRCCS Istituto Giannina Gaslini, UOC Genetica Medica, Milan, Italy, [4]University of Warwick, School of Life Sciences, Coventry, United Kingdom

Chronic bacterial infections affect individuals whose normal immune defenses are compromised, including those with cystic fibrosis (CF). *Pseudomonas aeruginosa* (*Pa*) colonization of the lower airways in people with cystic fibrosis (pwCF) leads to intractable biofilm infections. Despite repeated antibiotic administration, episodes of acute pulmonary exacerbation occur, eventually leading to death from respiratory failure. To counteract this type of antibiotic-refractory infection, phage therapy, the use of exogenous bacteriophages (or phages), is attracting increasing interest. In previous studies, we isolated and characterized a collection of phages able to kill *Pa in vitro*. A four-phage cocktail (CK φ) was able to counteract *Pa* acute infections in different animal models, although these models cannot reproduce some of the aspects of the CF airway, in particular concerning biofilm formation. Currently, most biofilm studies primarily rely on *in vitro* assays developed on abiotic surfaces, offering limited predictive value. Therefore, there is the need to develop models that can mimic the *in vivo* lung CF environment, thus reproducing the metabolic state of the pathogens.

Through this research, we have set up an *ex vivo* pig lung (EVPL) CF model, which closely mimics the physicochemical environment of human CF airways, allowing growth of *in vivo*–like biofilm. In this model, swine bronchial tissue is incubated with an artificial sputum medium that replicates the composition of CF airway fluids, allows for emulation of chronic CF pulmonary infections. We are investigating biofilm infections caused by various strains of *Pa*, including laboratory strains and strains obtained from pwCF who have been treated with modulators. The anti-biofilm potential of various phage preparations has been evaluating alone and in combination with antibiotics. We observed that phage treatment nearly eradicated biofilm formed by PAO1 and LESB58 strains in the EVPL model, also showing a synergistic effect with antibiotics, resulting in a decrease in the CFU count of biofilm biomass. Additionally, phage treatment disrupted biofilm architecture and strongly inhibited biofilm matrix production. The effectiveness of phages against biofilm growth in the EVPL model was weaker at equivalent concentrations compared to the in vitro biofilm developed on the Calgary device, suggesting the formation of a more robust biofilm in the EVPL model. Additionally, in the EVPL model the treatment with single phage preparation (DEV, *Schitoviridae*) partially eradicated the biofilm formed by *Pa* clinical isolates derived from PwCF. Furthermore, phage treatment was able to reduce intensity and spread of infection on bronchial tissue infected with the fluorescent reporter strain PAO1-GFP. A further validation of phage treatment will be performed using wild-type or CFTR mutated human primary bronchial epithelial cells.

The present study aims to demonstrate the effectiveness of the EVPL CF model in evaluating the efficacy of phages against *Pa* biofilm, addressing a critical challenge in phage therapy. The topic holds significant importance for PwCF, given the formidable resistance and persistence of *Pa*. Moreover, the implementation of a cost-effective, easy-of-use CF model has the potential to accelerate the translation potential of phage therapy into clinical practice.

P20

Utilisation of human induced pluripotent stem cells for modelling cystic fibrosis lung disease *in vitro*

M.-C. Jaboreck[1], A. Balázs[2], J. Zöllner[1], N. Cleve[1], L. Czichon[1], L. von Schledorn[1], J. Hegermann[3], J.C. Nawroth[4], D. Roth[4], M. Mielenz[5], S. Hedtfeld [5], F. Stanke[5], T. Rubil[2], F. Ius[6], D. Jonigk[7], J.W. Hanrahan[8], R. Olmer[1], M.A. Mall[2], S. Merkert[1], U. Martin[1]

[1]Hannover Medical School (MHH), Leibniz Research Laboratories for Biotechnology and Artificial Organs (LEBAO), Hannover, Germany, [2]Charité - Universitätsmedizin, Department of Pediatric Respiratory Medicine, Immunology and Critical Care Medicine, Berlin, Germany, [3]Hannover Medical School (MHH), Core facility for electron microscopy, Hannover, Germany, [4]Helmholtz Munich, Helmholtz Pioneer Campus and Institute of Biological and Medical Imaging, München, Germany, [5]Hannover Medical School (MHH), Clinic for Pediatric Pneumology, Allergology and Neonatology, Hannover, Germany, [6]Hannover Medical School (MHH), Transplantationszentrum, Clinic for Cardiothoracic-, Transplantation and Vasuclar Surgery, Hannover, Germany, [7]Universitätsklinikum Aachen, Reference Centre for Lung Pathology, Aachen, Germany, [8]McGill University, Department of Physiology, Montreal, Canada

Cystic Fibrosis (CF) is characterised by a complex multi-organ pathophysiology and lung disease as the major cause of mortality. Despite improved treatment options, the development of alternative drugs and individualised therapies is still urgently needed to address phenotypic variations and untreatable CFTR mutations. Intestinal organoids have been used as a CF model and personalised drug screening system but reflect CF lung disease only to a limited extent. Primary airway cells are useful, but only available in small quantities.
In contrast, patient-specific human induced pluripotent stem cells (hiPSCs), with their unlimited potential for proliferation and differentiation, and the possibility to use gene corrected clonal lines equipped with useful reporter transgenes, allow for the generation of organotypic culture systems comprising all relevant airway cell types. However, it has remained unclear to what extent such air-liquid-interface (ALI) cultures derived from hiPSCs recapitulate the pathophysiology of CF lung disease.
We now provide a comprehensive characterisation of hiPSC- derived airway (iALI) cultures and demonstrate the utility of iALI culture for CF research. Our data show a high similarity between iALI cultures and primary airway (pALI) cultures, the current gold standard model system. We compared mRNA and protein expression, mucus (ultra)structure and height, ion channel function and ciliary beat frequency to characterise the manifestation of the CF disease phenotype in iALI cultures and to test disease recovery by CFTR modulator treatment. The application of an automatable ciliary beat frequency assay, which is largely unaffected by common variable cellular impurities, facilitates sensitive measurement of mucociliary function as the major pathomechanism of CF lung disease. iALI cultures thus represent a valuable system for modelling CF lung disease and for personalised drug development with an unlimited supply of patient-specific cells.

P21

Development of new models to study the impact of CFTR dysfunction on osteocytes in cystic fibrosis-related bone disease

L. Thoraval [1], C. Guillaume[1], L. Hamon[1], G. Bouet-Chalon[2], A. Vanden-Bossche[3], D. Marchat[2], F. Velard[1]

[1]Université de Reims Champagne-Ardenne, UR BIOS, Reims, France, [2]Mines Saint-Etienne, Univ Jean Monnet, Etablissement Français du Sang, INSERM, U 1059 Sainbiose, Saint-Etienne, France, [3]Université Jean Monnet Saint-Étienne, INSERM, Mines Saint Etienne, U 1059 Sainbiose, Saint-Etienne, France

Objectives: Cystic fibrosis-related bone disease (CFBD) occurs in 20-50% of adults with cystic fibrosis (CF). CFBD patients present with low bone density and an increased risk of fractures, affecting their quality of life. Understanding the impact of CFTR defect on bone physiology is essential to propose more effective therapeutic strategies. Previous studies have already shown CFTR dysfunction effect on the activity of osteoblasts [Velard, 2014; 2015; Delion, 2016; Dumortier, 2025] and osteoclasts [Jourdain, 2021], leaving osteocytes out in the cold. Far from being just a passive placeholder in bone, osteocytes are long-lived cells within the bone matrix that perform a variety of functions, including the control of bone remodeling [Dallas, 2013]. Their abnormal behavior has been described as contributing to bone tissue deterioration in post-menopausal osteoporosis and skeletal aging [Jilka, 2016]. The aim of this work is, i) to develop an innovative *in vitro* model to study the impact of CFTR dysfunction on osteocytes in CF, ii) to analyze osteocytes *ex vivo* in CF(F508delCFTR) and non-CF mice, in an ovariectomy (OVX)-induced osteoporosis model.

Methods: Human primary mesenchymal stem cells (MSC) (n=3 independent donors) are cultured on textured bioceramics for 28 days [Jugnet, 2017], with or without CFTR pharmacological inhibitors (inh-172, BPO-27). Expression of osteocyte genes (*BGLAP*, *SOST*, *DMP1*, *FGF23*) is assessed by RT-qPCR and the associated protein secretion by ELISA. Stainings (Von Kossa, Hematoxylin Eosin, Masson's Trichrome) and fluorescent labeling (phalloidin-AlexaFluor®488, DAPI) are performed to identify mineralized and organic matrix production as well *in situ* cells morphology. Eight-week-old CF (n=7) or non-CF (n=6) mice undergo surgical ovariectomy (OVX) to mimic post-menopausal osteoporosis. Tibiae from control or OVX mice are collected, fixed in ethanol, decalcified and prepared for cryotomy and enhanced-resolution confocal analysis, or optical transparency for analysis by light-sheet microscopy.

Results: The first *in vitro* differentiation tests and *ex vivo* analyses are carried out to enable us to optimize the methods. Osteogenic medium as well as seeding conditions are optimized to obtain cells expressing osteocyte-markers at mRNA level. Impact of CFTR inhibitors on this osteocyte-like phenotype is currently under investigation. Analysis of fluorescent labeling samples with phalloidin-DAPI has demonstrated our ability to image *ex vivo* osteocytes and their cytoplasmic extensions using confocal microscopy. We successfully perform OVX surgery (n=7 CF and n=6 non-CF mice), evidencing blunted bone microarchitecture in CF condition worsen by OVX. In addition, staining of *ex vivo* samples and their analysis by microscopy will reinforce our understanding of osteocyte phenotype and osteocyte lacuna-canalicular network structure in CF *vs* non-CF conditions.

Conclusion: Future work using iPSC-derived MSC will allow us to gain insights on native CFTR mutation effect in comparison with functional inhibition of the channel to shed light on the involvement of osteocytes in the deregulation of bone homeostasis in CF patients, reinforcing this work evidence that CFTR loss of function in CF induced global dysregulation of all bone cells.

P22

Pancreatic cancer growth rate may be susceptible to variations in CFTR expression

R. Ibáñez[1], J.P. Muñoz[1], M. Corbacho[2], J. Barquinero[3], M.T. Salcedo[4], L. Campderros[1], E.C. Vaquero[2], X. Molero[1]

[1]Vall d'Hebron Institute of Reserach (VHIR), Pathophysiology and treatment of pancreatic disorders, Barcelona, Spain, [2]ICMDM, Hospital Clínic, IDIBAPS, Gastroenterology Department, Barcelona, Spain, [3]Vall d'Hebron Institute of Reserach (VHIR), Gene and Cell Therapy Department, Barcelona, Spain, [4]Hospital Universitari Vall d'Hebron, Pathology Department, Barcelona, Spain

Background: Cystic Fibrosis patients are at higher risk of developing cancer of the gastrointestinal tract that shows accelerated growth. Pancreatic stellate cells (PSCs) are active members of the cancer microenvironment. Moreover, subcutaneous injection of pancreatic cancer cells along with PSCs results in increased tumour growth. We have shown CFTR is expressed and regulated in PSCs, and reduced CFTR expression is linked to the acquisition of a proliferative and proinflammatory phenotype. Reduced expression of CFTR may contribute to generation of more aggressive pancreatic tumours.

Methods: CFTR expression was examined in human pancreatic cancer cell lines MiaPaca2, Panc-1, Capan2 and BxPC3, and in murine pancreatic cancer cells KPC (K-ras and p53 mutated) by means of ddPCR. Cancer cell lines were ranked from more aggressive to less aggressive according to their doubling proliferation time rate and differentiation grade (E-cadherin/Zeb-1 expression). PSCs were isolated from wild-type (wt) or CFTR-KO mice and their proliferation rate examined. The proliferation of luciferase-expressing KPC cells was examined in stand alone cultures, or after being co-cultured with either wt-PSC or CFTR-KO PSC. Allogeneic subcutaneous pancreatic tumours were induced in normal mice by injecting a mixture of KPC and PSCs (either wild-type or CFTR-KO) in Matrigel /DMEM media. Tumour growth was monitored for 28 days. Before sacrifice, mice were submitted for computerized tomography examination to extract radiomic features. Tumours were then excised, weighted and processed for histopathology.

Results: Despite being of "ductal" origin, human pancreatic ductal adenocarcinoma cell lines expressed very low levels of CFTR. CFTR expression was inversely associated with the degree of differentiation, being undetected in MiaPaca2 cells, barely expressed in Panc-1/Capan2 cells, and better detected in BxPC3 cells. The well-differentiated murine pancreatic cancer cell line KPC showed the largest CFTR expression. PSCs devoid of CFTR (CFTR-KO PSCs) showed higher rates of proliferation than wild-type PSCs. In addition, pancreatic cancer cells KPC showed higher proliferation rates when co-cultured with CFTR-KO PSCs, as compared to KPCs co-cultured with wild-type PSCs or to KPC cultured alone. Pancreatic tumours generated using CFTR-KO PSCs had larger volumetric growth rate and final weight than tumours developed using wild-type PSCs. Pathology examination disclosed greater disorganized structure in tumours from CFTR-KO PSCs that showed enhanced radiomic features associated with tumour aggressiveness (lesser entropy, contrast and cluster tendency, and higher energy and nonuniformity), which supported the pathology findings. In summary, reduced CFTR expression matches cell dedifferentiation and proloiferation rates in pancreatic cancer cell lines, and PSCs defective in CFTR enhance pancreatic cancer growth, both in vitro and in vivo.

Conclusion: CFTR expression levels impacts on pancreatic cancer growth rate and on the tumour inner structure. Global CFTR activity may be a relevant factor modulating cancer aggressiveness.

P23

Does CFTR Play a Role in Epithelial Differentiation, EMT and Cancer?

C.S Rodrigues, I. Pankonien , V. Railean, M.D Amaral

BioISI—Biosystems & Integrative Sciences Institute, Faculty of Sciences, University of Lisboa, Lisboa, Portugal

Background: Recent studies suggest that CFTR plays a role in diverse cellular processes, such as epithelial differentiation, polarization, and tissue regeneration [1]. Furthermore, a higher risk of developing several types of cancer has been observed among people with CF (pwCF) with a risk for colorectal cancer 3.5-fold higher than the general population. Other studies reported that CFTR functions as a tumour suppressor contributing to keep proliferation under control. Additionally, epithelial-to-mesenchymal transition (EMT) was found to be active in CF cells and tissues [2]. However, the mechanisms by which CFTR regulates all these processes are still poorly understood.
Objective: To explore the role of CFTR in epithelial differentiation, EMT and cancer.
Methods: Human CF bronchial epithelial cells (CFBE) expressing p.Phe508del- or p.Gly551Asp-CFTR were analysed for epithelial and mesenchymal markers by Western Blot (WB) and immunofluorescence (IF) and Transepithelial electrical resistance (TEER) was measured. The BCi-NS1.1 basal cell line [3] was edited by CRISPR/Cas9 to generate two novel human airway basal cell lines (BCi-CF1.1 and BCi-CF1.2) to express the above CFTR variants in homozygosity. Their differentiation into different cell types, and epithelial/mesenchymal markers were assessed, and CFTR function was measured by Ussing chamber. Intestinal organoids generated from healthy and CF donors were differentiated, and analysed for epithelial and mesenchymal markers.
Results: Data in CFBE cells show that the expression of the mesenchymal markers N-cadherin and vimentin are increased only in p.Phe508del-CFTR expressing cells vs wt-CFTR cells but not in p.Gly551Asp-CFTR cells. Additionally, TEER values are significantly decreased in both CF cell types vs wt-CFTR cells. However, p.Phe508del-CFTR cells show lower TEER values than p.Gly551Asp-CFTR cells. Furthermore, WB and IF results reveal that BCi-NS1.1, BCi-CF1.1 and BCi-CF1.2 cells differentiate into the various airway cell types. Functional analyses revealed a typical CFTR-mediated chloride secretion in BCi-NS1.1 cells, but not in CF cells. Moreover, CFTR modulators rescued CFTR function in the two CF cell lines. Further studies are underway to understand how each of these CFTR variants impact airway epithelial differentiation and regeneration. Undifferentiated CF and non-CF intestinal organoids express various epithelial and mesenchymal markers, including ZO-1, E-cadherin, claudin-1, cytokeratin-18, N-cadherin and vimentin. The same markers will be assessed on organoids currently being differentiated.
Conclusion: The results suggest that differentiation of CFBE cells expressing dysfunctional but PM located p.Gly551Asp-CFTR (class III) is improved vs differentiation of p.Phe508del-CFTR which is retained in the ER, not reaching the PM (class II). These two novel basal CF cells constitute unique models to investigate the role of CFTR in cell-type differentiation and regeneration.
Acknowledgements: Work supported by UID/04046/2025 centre grant (to BioISI), from FCT/MCTES Portugal, and research grants “HIT-CF” (H2020-SC1-2017–755021) from EU; CR and VR are recipients of PhD fellowships SFRH/BD/153053/2022 from BioSys PhD programme PD/00065/2012 and SFRH/BD/06718/2021, respectively. and IP of contract DOI 10.54499/DL57/2016/CP1479/CT0013, all from FCT (Portugal).

[1] Amaral *et al* (2020) *Int J Mol Sci* **21**: 3133.
[2] Quaresma *et al* (2022) *Life Sci Alliance* **5**: e202101326; Quaresma *et al* (2020) Cell Death & Dis **11**: 920.
[3] Walters *et al* (2013) *Respir Res* **14**(1):135

P24

Key role of bone marrow adiposity in the development of Cystic Fibrosis-related Bone Disease

L. Hamon, C. Dumortier[1,2], L. Thoraval[1], J. Sergheraert[1,3], O. Piot[4], E. Buache[4], N. Goffin[4], C. Chauveau[5], S. Gangloff[1], D. Al Alam[2], M.-L. Jourdain[1,3], F. Velard[1]

[1]Université Reims Champagne-Ardenne, UR BIOS, Reims, France, [2]The Lundquist Institute, Harbor-UCLA Medical Center, Torrance, United States, [3]CHU de Reims, Service de Médecine Bucco-Dentaire, Hôpital Maison Blanche, Reims, France, [4]Université Reims Champagne-Ardenne, UR BioSpeCT, Reims, France, [5]Université Littoral Côte d'Opale, ULR 4490 MabLab, Boulogne-sur-Mer, France

Cystic Fibrosis-related Bone Disease (CFBD) affects 50% of CF adult patients. An alteration in bone formation and maturation has been demonstrated, but remains poorly understood (Velard, 2014). Osteoblasts obtained from CF induced pluripotent stem cells (iPSCs) evidenced a higher PPARG level (adipocyte transcription factor) than healthy control line (Dumortier, 2025). A relationship exists between low bone density and increased medullar adiposity, (Rozman, 1989 ; Verma, 2002 ; Zhou, 2008 ; Piccinin, 2014) so we hypothesized that there is a preferential adipocyte differentiation triggered in mesenchymal stem cells (MSC) bearing mutation in *CFTR* gene, which causes CF. To date, medullar adiposity has never been investigated in CFBD.

MSC from healthy donors have been differentiated into adipocytes and osteoblasts during 21 days, with or without Inh_{172} or BPO27 (CFTR pharmacological inhibitors) (n=7). Oil Red'O, Bodipy™, FABP4 and PPARG (adipocytes specific proteins) stainings were performed. Raman microspectroscopy was used to determine the nature of lipid content. MicroCT analysis of WT and F508delCFTR mice who underwent surgical ovariectomy (OVX) or not (sham) was performed at 8 and 16 weeks post-OVX to assess bone microarchitecture.

Blocking CFTR had a direct effect on adipocyte number and by increasing their specific markers expression and lipid vesicles size (n=7 donors, $p<0.05$). Raman analyses showed an increased saturation degree of lipids. More, lipid droplets spontaneous generation was evidenced in MSC and osteoblasts with both CFTR inhibitors. This may reflect a prefered adipocyte commitment due to impaired CFTR function. MicroCT data show a decreased trabecular thickening in CF mice compared to WT mice, leading to the alteration of bone microarchitecture in these CF mice. We also show a trend towards a decrease in bone mineral density during OVX CF mice. In addition, the number of bone trabeculae decreased in contrast to the trabecular separation that increased in OVX CF mice compared to CF mice.

These data are the first proof of concept that CFTR loss of function influences size and content of mature adipocytes lipid vesicles, as well as MSC commitment towards adipocyte lineage. We evidenced a more pronounced manifestation of CFBD in OVX mice, thus highlighting the potential impact of post-menauposis on female CF patients. Finally, understanding the formation and role of lipid metabolism in this context may be a promising avenue to better understand and cure CFBD.

P25

Elexacaftor/tezacaftor/ivacaftor CFTR modulators mitigate senescence in cystic fibrosis

A.M. Hristodor[1], T. Gunawardena[2], M. Borgatti[3], A. Vella[4], C. Boni[1], D. Olioso[5], F. Quiri[1], G. Lippi[5], T. Moraes[6,7], M. Cipolli[1], V. Bezzerri[1,8]

[1]Azienda Ospedaliera Universitaria Integrata di Verona, Cystic Fibrosis Center, Verona, Italy, [2]Hospital for Sick Children, Department of Molecular Medicine, Research Institute, Toronto, Canada, [3]University of Ferrara, Department of Life Sciences and Biotechnology, Ferrara, Canada, [4]Azienda Ospedaliera Universitaria Integrata di Verona, Unit of Immunology, Verona, Canada, [5]University of Verona, Section of Clinical Biochemistry, Department of Engineering for Innovation Medicine, Verona, Italy, [6]Hospital for Sick Children, Division of Respiratory Medicine, Department of Paediatrics, Toronto, Canada, [7]Hospital for Sick Children, Department of Translational Medicine, Research Institute, Toronto, Canada, [8]Link Campus University, Department of Life Sciences, Health, and Health Professions, Rome, Italy

Impaired CFTR function causes loss of chloride and bicarbonate efflux across epithelia, determining airway surface liquid dehydration, oxidative stress, and mucus accumulation into the bronchial lumen. This microenvironment promotes subsequent bacterial infections, mainly sustained by *P. aeruginosa*, that amplify the inflammation.

Senescence has been initially described as a state of irreversible cell cycle arrest induced as a response to oxidative stress, DNA damage, or physiologic aging. Senescence may act as a double-edged sword, showing both beneficial and detrimental effects. In the short term, senescence acts as a protective mechanism, supporting wound healing and preventing damaged cells from proliferating. On the other hand, the chronic accumulation of senescent cells releasing proinflammatory factors, collectively referred to as the Senescence-Associated Secretory Phenotype (SASP), further contribute to low-grade chronic inflammation. This leads to the "inflammaging" process, increasing the risk of age-related disorders, fibrosis, cancer, and autoimmune diseases. Thus, we sought to clarify if senescence plays a relevant role in CF pathophysiology, representing a main driving-force of the constitutional inflammatory vicious cycle. Then, we evaluated the effect of elexacaftor/tezacaftor/ivacaftor (ETI) on the expression of several senescence markers in various CF airway epithelia.

Normal and CF bronchial epithelial cell lines, as well as primary air-liquid interface (ALI)-differentiated human bronchial (hBECs) and nasal (hNECs) epithelial cells, homozygous for the F508del CFTR variant, with similar donor ages and culture passages, were used. Immunofluorescence, cell stress proteome profiling array (R&D, Minneapolis) and western blot were used to evaluate senescence biomarkers in protein extracts from hBECs and hNECs. We found elevated levels of tumor suppressor p53 and its downstream effectors p21 and p16 in CF bronchial epithelia. Interestingly, the inhibition of CFTR-dependent chloride efflux sustained by CFTR(inh)-172 further increased p53 and p16 levels in healthy hBECs. Several stress-related proteins linked to senescence, such as COX-2, Bcl-2, HSP27, HSP60, p-JNK, and NF-κB, were elevated in CF hBECs. Higher levels of SASP-related cytokines and chemokines (IL-8, IL-1Rα, IP-10, GM-CSF) were also noted. Morphologically, CF bronchial epithelial cells were enlarged and flattened, showing increased expression of cytoskeletal component vimentin and decreased Lamin B1 compared to non-CF cells, according to the senescence hypothesis.

Most importantly, while CFTRinh-172 induced vimentin expression in healthy hNECs, ETI restored normal levels of vimentin and Lamin B1 in CF hNECs by modulating p53 pathway.

Of note, SASP soluble mediators were analyzed in plasma samples from 50 patients with CF (homozygous for F508del mutation) before and after 12 months of ETI therapy. Plasmatic levels of several SASP mediators (IL-1β, IL-6, IL-8, G-CSF, MIP-1α, TNFα, and IL-17) were significantly reduced upon ETI therapy.

These results highlight that: i) senescence biomarkers are constitutively elevated across various CF airway models: ii) senescence may act as a major driver of the constitutive inflammation observed in CF: iii) ETI can partially mitigate senescence biomarkers both in vitro and in vivo, highlighting potential benefits that may go far beyond the expected ones.

P26

Selective impairment of CFTR-associated bicarbonate transport is recovered by CFTR modulators in vitro in an individual carrying the F508del/L997F CFTR genotype affected by recurrent pancreatitis

K. Kleinfelder[1], U. Krivec[2,3], P. Pascolo[2], A.P. Novak[4], F. Amato[5], A.M. Hristodor[6], M. Bertini[6], E. Pintani[6], P. Melotti[6], C. Sorio[1]

[1]University of Verona, Department of Medicine, Verona, Italy, [2]University Children's Hospital Ljubljana, University Medical Centre Ljubljana, Paediatric Pulmonology, Ljubljana, Slovenia , [3]University of Ljubljana, Faculty of Medicine, Ljubljana, Slovenia, [4]University Children's Hospital Ljubljana, Gastroenterology, Hepatology, and Nutrition, Ljubljana, Slovenia, [5]University of Naples Federico II and CEINGE-Advanced Biotechnologies, Department of Molecular Medicine and Medical Biotechnologies, Naples, Italy, [6]Azienda Ospedaliera Universitaria Integrata, Cystic Fibrosis Centre, Verona, Italy

Patients with idiopathic recurrent-acute or chronic pancreatitis diagnosed as CFTR-related disorder harbor one or two pathogenic CFTR variants and/or present reduced levels of CFTR function. There is no specific therapy for pancreatitis. Here, we describe a case of a Slovenian girl carrying F508del in trans with L997F CFTR variants and polymorphism IVS8:T9/T9 with recurrent pancreatitis. She was referred to the CF Center of Verona in Italy, where the standardized CFTR measurements, such as nasal potential differences (NPD) and intestinal current measurements (ICM), were in the normal range while presenting sweat test borderline values (Cl^- 48-55 mmol/L). Her optical beta-adrenergic sweat test was also normal. Given the importance of bicarbonate transport in the pathophysiology of the pancreas, we assessed whether there was a selective deficiency in its CFTR-dependent transport in this case. Hence, we evaluated CFTR-dependent chloride or bicarbonate transport separately, along with the effect of ELX/TEZ/IVA (ETI) across patient-derived rectal organoids in the Ussing chamber. Non-CF and CF F508del/F508del organoids were used as reference.

Our results demonstrated that in the absence of HCO_3^-, F508del/L997F rectal organoid-derived epithelial monolayers present good CFTR activity elicited by forskolin (50±8 μA/cm²) close to the non-CF range (91±30 μA/cm²). We measured the CFTR-mediated current for the reference genotype F508del/F508del, strongly associated with pancreatic insufficiency, which had a severely reduced response in chloride-only buffer, representing less than 5% of anion conductivity of non-CF (4±2 μA/cm²). ETI-rescued F508del-CFTR improved Cl^- secretion (40±13 μA/cm²). Interestingly, F508del/L997F Cl^- currents remained unaffected (50±23μA/cm²) upon ETI treatment. Having HCO_3^- as a CFTR permeable ion, currents were strongly impaired (4±3 μA/cm²) for F508del/L997F as it was for the F508del/F508del (1±0.3 μA/cm²) in comparison to the non-CF group (29±11 μA/cm²). ETI treatment positively affected HCO_3^- transport in the F508del/L997F case (12±4 μA/cm²), reaching 41% of the conductivity seen in the non-CF control, a level similar to ETI-rescued F508del/F508del organoid-derived intestinal monolayers (10±2 μA/cm²).

The beneficial effect of ETI in ameliorating F508del/L997F-CFTR function was also recorded in patient-derived rectal organoids and nasal epithelial cells using a buffer that could not discriminate the individual contribution of these anions. In conclusion, F508del/L997F colonoids, while presenting with an overall conserved CFTR anion transport, seem to have a selective defect in CFTR-dependent HCO_3^- transport that ETI treatment can significantly correct. Even if we could not directly access pancreatic tissue, it is tempting to speculate that, given the relevance of this anion in pH maintenance and acidification of the pancreatic juice, its severe reduction associated with this specific genotype may be associated with an increased risk of developing pancreatitis. Our data demonstrate the importance of studying CFTR in primary cells, specifically patients with episodes of pancreatitis associated with CFTR variants, to better characterize possible molecular defects. In particular, assessing these anions separately lets us better define CFTR dysfunction. It may lay the basis to guide the scientific community in evaluating personalized therapies that may prevent further disease aggravation or the development of other disorders associated with specific CFTR variants.

P27

Characterization of a frameshift variant by means of patient-derived nasal epithelial cells: molecular and functional analysis of *CFTR* mRNA and protein

C. Pastorino[1], V. Capurro[1], V. Tomati[1], E. Pesce[1], M. Lena[1,2], A. Dighero[1], L. Menta[1,2], R. Bocciardi[1,2], N. Pedemonte[1]

[1]IRCCS Istituto Giannina Gaslini, UOC Genetica Medica, Genoa, Italy, [2]University of Genoa, Department of Neurosciences, Rehabilitation, Ophthalmology, Genetics, Maternal and Child Health (DINOGMI), Genoa, Italy

Cystic Fibrosis (CF) is caused by variants in the *CFTR* gene which can compromise the function of the CFTR protein through different molecular mechanisms.
CFTR modulators have been developed for the rescue of the basic functional defect(s) of different variants. However, many individuals with CF carry variants which are orphan of treatment (in Italy about 30%).
In the frame of a project aimed at characterizing rare variants, we have recruited so far seven patients compound heterozygous for the frameshift variant 4382delA (c.4251del; p.Glu1418ArgfsX14) and a minimal function, not-rescuable variant (resembling a null allele). The 4382delA variant (which has a 0.74% allelic frequency in Italy) is localized on the last exon of the *CFTR* gene and it has been reported that, on cell lines, there is the synthesis of a normal protein up to the aminoacid in position 1417, with an additional tail of 15 aminoacids, followed by the Premature Termination Codon (PTC). This abnormal protein displays a functional defect, which has been shown to be rescued by pharmacological modulators [DOI: 10.1371/journal.pgen.1007723]. In addition, the corresponding mRNA could be detected in patient-derived nasal cells, suggesting that it does not undergo Nonsense-Mediated Decay (NMD) [DOI: 10.1371/journal.pgen.1007723].
In this work, we present preliminary data on the characterization of the 4382delA variant using patient-derived nasal cells, with the aim of defining/providing a personalized therapeutic approach.
Patient's cells from nasal brushing were cultured, expanded and reprogrammed to generate differentiated epithelia. Then, CFTR function was evaluated in Ussing Chamber upon treatment with different CFTR modulators. Cells were then harvest for RNA extraction to allow studies of *CFTR* mRNA.
Short-circuit current analysis on patients epithelia highlighted, overall, a reduced CFTR activity under basal condition, although, we observed some interindividual variability in its extent. Interestingly, following treatment with modulators, we consistently observed that total CFTR was increased, with an improvement of about 4-7 percent point (with respect to total CFTR activity). For all the patient-derived cultures, rescued CFTR-mediated current was equal to 3-5 $\mu A/cm^2$, corresponding to 10-15% of normal CFTR current.
We are now evaluating the possible correlation among CFTR basal activity, its pharmacological rescue and *CFTR* mRNA expression levels (both total and from single alleles).
Further characterization of mutant protein rescue by novel modulators is also ongoing.
This work allowed us to study the functional and molecular mechanisms caused by the rare 4382delA variant in a native context, highlighting the crucial value of patient-derived cells and possibly supporting new therapeutic options for patients carrying this variant.

Supported by Fondazione Ricerca Fibrosi Cistica grants FFC #9/2019 and FFC #10/2021, Italian Ministry of Health grants GR-2018–12367126 and PNRR-MR1-2023-12378412 and Cystic Fibrosis Foundation grant PEDEMO20G0.

P28

Investigating the safety of Trikafta/Kaftrio exposure during early development

A. Squarzoni[1], S.M. Bertozzi[1], M. Summa[1], R. Bertorelli[1], T. Bandiera[1], G. Capodivento[2], C. Montani[2], V. Tomati[3], V. Capurro[3], N. Pedemonte[3], F. Benfenati[4], L. Nobbio[5], A. Armirotti[1]

[1]Istituto Italiano di Tecnologia (IIT), Genova, Italy, [2]IRCCS Ospedale Policlinico San Martino, Neuropathology Laboratory, UO Clinica Neurologica, Genova, Italy, [3]Istituto Giannina Gaslini, U.O.C. Genetica Medica, Genova, Italy, [4]IRCCS Ospedale Policlinico San Martino, IIT Center for Synaptic Neuroscience and Technology - Advanced Biotechnology Center, Genova, Ital, [5]IRCCS Ospedale Policlinico San Martino, Centro di Biotecnologie Avanzate (CBA), Genova, Italy

Elexacaftor/Tezacaftor/Ivacaftor (ETI) can largely extend the lifespan of people with CF (pwCF) and improve their quality of life (1). While the use of this drug has not been approved during pregnancy, pregnant CF women frequently choose to continue ETI treatment (2). Although no severe side effects on fetuses/newborns have been reported so far in literature (2), previous data from our laboratory show that Tezacaftor is an inhibitor of DEGS, the desaturase that converts dihydroceramides (dHCer) into Ceramides (Cer) (3). Given the role of this enzyme in the central and peripheral nervous systems (PNS, CNS, 3,4,5), we aim at ruling out potential long-term side effects of the use of ETI during the development of CNS and PNS, by conducting a long-term exposure study in mice.

To evaluate the effect of ETI during pregnancy and breast feeding, we will expose CD-1 female mice to ETI through food. After three weeks of pre-exposure, the female will be allowed to mate and, for the whole duration of pregnancy and breastfeeding, exposure to ETI will continue. We will conduct a set of molecular analyses, assessing the levels of ETI in mum's and pup's blood and tissues and we will measure the potential accumulation of dHCer. Their neurological abilities and general wellbeing will also be assessed with behavioural tests. Finally, further morphological and functional assessment of PNS and CNS functionality will be carried out.
We are currently setting up all the tools needed for the animal experiments (starting Jan 2025), including an advanced micro sampling method to quantify circulating ETI in pups.
We believe that this project, aiming at detecting potential early side effects of ETI on neurodevelopment during pregnancy and breastfeeding, is of relevance for the safe and informed use of ETI by women with CF.

References:

1. Heijerman HGM, McKone EF, Downey DG, et al. Efficacy and safety of the elexacaftor plus tezacaftor plus ivacaftor combination regimen in people with cystic fibrosis homozygous for the F508del mutation: a double-blind, randomised, phase 3 trial [published correction appears in Lancet. 2020 May 30;395(10238):1694. doi: 10.1016/S0140-6736(20)31021-7]. Lancet. 2019;394(10212):1940-1948. doi:10.1016/S0140-6736(19)32597-8
2. Taylor-Cousar JL, Jain R. Maternal and fetal outcomes following elexacaftor-tezacaftor-ivacaftor use during pregnancy and lactation. J Cyst Fibros. 2021;20(3):402-406. doi:10.1016/j.jcf.2021.03.006
3. Liessi N, Tomati V, Capurro V, et al. The combination elexacaftor/tezacaftor/ivacaftor (ETI) modulates the de novo synthethic pathway of ceramides in a genotype-independent manner. J Cyst Fibros. 2023;22(4):680-682. doi:10.1016/j.jcf.2023.04.012
4. Ciobanu DZ, Liessi N, Tomati V, et al. Tezacaftor is a direct inhibitor of sphingolipid delta-4 desaturase enzyme (DEGS). J Cyst Fibros. 2024;23(6):1167-1172. doi:10.1016/j.jcf.2024.05.004
5. Doretto S, Malerba M, Ramos M, et al. Oligodendrocytes as regulators of neuronal networks during early postnatal development. PLoS One. 2011;6(5):e19849. Published 2011 May 12. doi:10.1371/journal.pone.0019849

P29

GoSlo, a Potassium Channel Modulator, Enhances CFTR Activity in Wild-Type and F508del-CFTR Variants

J.N. Lunavath, G.W.J. Moss, P. Vergani

University College London, Neuroscience, Physiology and Pharmacology, London, United Kingdom

CFTR modulators target the protein at the root of cystic fibrosis (CF). While drugs such as elexacaftor, tezacaftor, and ivacaftor (ETI) have transformed treatment for many people with CF, there remains a critical need for therapies to treat people who cannot benefit from current CFTR modulators. Potassium channel activators have been considered, as they could increase the driving force for anion efflux, through CFTR or alternative pathways. Surprisingly, we find that GoSlo-SR-5-6 (GoSlo), a potassium channel modulator[1], directly targets CFTR, increasing ion-channel function in both wild-type (WT) and F508del-CFTR.

HEK293 cells were transiently transfected with pIRES2-mCherry-YFP-CFTR plasmids (WT or F508del variants) and subjected to a high-content image-based assay[2]. Fluorescence quenching post iodide addition informed on CFTR-mediated anion flow (using Area Above the quenching Curve, AAC7). Biogenesis was quantified as CFTR membrane proximity[2]. GoSlo (10 µM) was applied chronically (24 hours) to cells expressing both WT- and F508del-CFTR, in the presence or absence of ETI (3 µM, 3 µM, 0.5 µM)

In WT-CFTR-expressing cells, GoSlo significantly increased CFTR function compared to DMSO controls ($p = 0.001$, paired t-test, $n=4$), demonstrating that this potassium modulator can enhance baseline CFTR activation. In cells expressing F508del-CFTR, GoSlo further amplified CFTR activity when combined with ETI ($p=0.012$, paired t-test, $n=4$). This enhancement was observed in both WT and F508del backgrounds. One possible interpretation is that GoSlo may activate CFTR via molecular mechanisms distinct from those of other modulators. No negative effects on CFTR biogenesis were detected.

Further investigations into the mechanism by which GoSlo influences CFTR activity are planned, with future studies including work on primary airway epithelial models and patient-derived organoids.

References:

1. Webb et al., 2015, PNAS 112(7):2064-2069
2. Prins et al., 2020, JBC 295(49):16529-16544

genotype, chronic treatment	AAC7	n
WT-CFTR, DMSO	0.60 ± 0.12	4
WT-CFTR, GoSlo 10 µM	1.10 ± 0.14	4
F508del-CFTR, ETI 3 µM, 3 µM, 0.5 µM	0.50 ± 0.07	4
F508del-CFTR, ETI 3 µM, 3 µM, 0.5 µM + GoSlo 10 µM	0.96 ± 0.03	4

P30

In silico, in vitro and ex vivo characterization of CFTR pathogenic variants localized in the Fourth Intracellular Loop and their rescue by modulators

E. Pesce [1], V. Tomati[1], V. Capurro[1], M. Lena[2], C. Pastorino[1], M. Astore[3], S. Kuyyucak[3], B. Chevalier[4,5], E. Sondo[1], F. Cresta[6], V. Terlizzi[7], S. Costa[8], M.C. Lucanto[8], V. Daccò[9], L. Claut[9], F. Ficili[10], R. Bocciardi[1,2], F. Zara[1,2], C. Castellani[6], L.J. Galietta[11], S.A. Waters[12,13,14], A. Hinzpeter[4,5], N. Pedemonte[1]

[1]IRCCS Istituto Giannina Gaslini, UOC Genetica Medica, Genova, Italy, [2]University of Genoa, Department of Neurosciences, Rehabilitation, Ophthalmology, Genetics, Maternal and Child Health (DINOGMI), Genova, Italy, [3]School of Physics, University of Sydney, Sydney, Australia, [4]Institut Necker Enfants Malades, INSERM, CNRS, Paris, France, [5]Université Paris-Cité, Paris, France, [6] IRCCS Istituto Giannina Gaslini, UOSD Centro Fibrosi Cistica, Genova, Italy , [7]Meyer Children's Hospital IRCCS, Cystic Fibrosis Regional Reference Center, Department of Pediatric Medicine, Firenze, Italy, [8]Azienda Ospedaliera Universitaria Policlinico G. Martino, Centro Hub Fibrosi Cistica, Messina, Italy 8, [9]Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico, Department of Pediatrics, Cystic Fibrosis Center, Milano, Italy 9, [10]Ospedale Giovanni Di Cristina, Cystic Fibrosis Center, Palermo, Italy, [11]Telethon Institute of Genetics and Medicine (TIGEM), Pozzuoli, Italy, [12]Faculty of Medicine and Health, School of Clinical Medicine, Discipline of Paediatrics and Child Health, Sydney, Australia, [13]Molecular and Integrative Cystic Fibrosis Research Centre, Sydney, Australia, [14] Sydney Children's Hospital, Department of Respiratory Medicine, Sydney, Australia

Cystic fibrosis (CF) is due to loss-of-function variants of the CFTR chloride/bicarbonate channel. The most effective treatment for people with CF (pwCF) carrying the F508del mutation (accounting for 70% of the pathogenetic alleles) is the triple combination of Elexacaftor-Tezacaftor-Ivacaftor (ETI). Although initially developed for F508del, CFTR modulators can correct the underlying defect(s) in other CFTR mutants. While in the USA, ETI has been approved for 271 mutations other than F508del, in Europe, its approval is limited to F508del. The use of disease-relevant predictive models such as patient-derived human nasal epithelial cells (HNEC) allow to investigate the response to CFTR modulators of specific genotypes, possibly supporting patients' access to treatment.

Previous studies have investigated the impact of several missense variants within the Intracellular Loop 4 (ICL4) on CFTR protein maturation and channel activity. While some of these variants, such as R1066H or L1077P, are already deemed eligible for ETI therapy, others, such as L1065P and R1066C, remain poorly investigated. Given their frequency in Italy and other Southern European countries, we aimed to collect evidence to characterize their functional defects and demonstrate their rescue by CFTR modulators in disease-relevant cell models. To this aim, we performed a detailed analysis of selected variants in the ICL4 using computational, functional and biochemical methodologies, to understand their impact on CFTR structure and function. Mutations affecting L1065, R1066, and L1077 result in disruptions of hydrophobic interactions, salt bridges, and helical integrity, respectively, leading to compromised structural stability of CFTR. Among the mutations analyzed, L1065P appears to induce the most significant conformational change, characterized by increased flexibility and loss of helix formation within the hydrophobic pocket, while the disruption of the salt bridge by R1066C and the helical destabilization by L1077P further underscore the role of ICL4 in maintaining CFTR's conformational stability. Analyses of single variants expressed heterologously in immortalized bronchial cells showed that, upon ETI, rescued activity for both L1065P and R1066C was close to 50% of the wild-type CFTR activity. Biochemical studies of ICL4 variants expression pattern in CFBE41o- cells, following treatment for 24 h, demonstrate the appearance of the band C, corresponding to mature, fully glycosylated protein, with no changes in the immature band. Cell surface measurements of CFTR performed using the HiBiT complementation assay in transiently transfected HEK293 cells confirmed partial restoration of CFTR surface expression, in the same range as observed with F508del. Finally, our study provides evidence in primary nasal cells from a cohort of pwCF that L1065P and R1066C, can be effectively rescued by ETI. Upon treatment with modulators, the CFTR-mediated current averaged around 25%-45% of the activity measured in non-CF epithelia. Although the observed rescue for L1065P and R1066C was smaller than that of the F508del (40-65% of the normal activity), it should fall in a range predicted, by various studies, to provide a clinical benefit.

Supported by Fondazione Ricerca Fibrosi Cistica grants FFC #9/2019 and FFC #10/2021, Italian Ministry of Health grants GR-2018–12367126 and PNRR-MR1-2023-12378412 and Cystic Fibrosis Foundation grant PEDEMO20G0.

P31

Deleterious effect of *P. aeruginosa* on F508del-CFTR rescued by Elexacaftor/Tezacaftor/Ivacaftor is clinical-strain dependent in patient-derived nasal cells

C. Allegretta[1], E. Montemitro[2], M.N. Sgobba[3], V. Capurro[4], E. Pesce[4], F. Ciciriello[2], G. La Bella[1], M. Rossito[2], V. Tuccio[2], F. Arena[1], T.N. Gunawardena[5], L. Guerra[3], N. Pedemonte[4], N. Capitanio[1], C. Piccoli[1], O. Laselva[1]

[1]University of Foggia, Department of Clinical and Experimental Medicine, Foggia, Italy, [2]Bambino Gesù Children's Hospital, Cystic Fibrosis Center, Specialistic Pediatrics Department, Rome, Italy, [3]University of Bari, Department of Bioscience, Biotechnology and Biopharmaceutics, Bari, Italy, [4]IRCCS Istituto Giannina Gaslini, UOC Genetica Medica, Genova, Italy , [5]5The Hospital for Sick Children, Programme in molecular Medicine, Toronto, Canada

Background: The triple cystic fibrosis transmembrane conductance regulator (CFTR) modulators combination Elexacaftor/tezacaftor/ivacaftor (ETI) has been approved for people with CF (pwCF) bearing at least one *F508del* allele. Despite the development of CFTR modulators having dramatically improved respiratory outcomes in pwCF, clinical studies have showed variable responses to this drug formulation. Of note, airway inflammation and bacterial colonization persist in the upper and lower respiratory tract even in ETI treated patients. Moreover, reactive oxygen species (ROS)have been shown to interfere with CFTR function[1, 2] and treatment with anti-oxidants like glutathione rescued LPS-decreased CFTR function [3].
Methods: To measure the effect of exposure to *P. aeruginosa* EXOs on CFTR function, we employed fluorescence-based membrane potential assay (FMP) and Ussing chamber assay. Pro-inflammatory cytokines were measured by RT-PCR and ELISA kits and ROS levels by ROS sensitive fluorescence probe (DCF).
Results: we first tested the clinical exoproducts (EXO) of *P. aeruginosa* isolated from 15 CF patient in WT and F508del-CFTR CF bronchial epithelial (CFBE) cells and found that EXO variably decreased WT-, F508del- and ETI-dependent F508del-CFTR function and increased proinflammatory cytokines and ROS levels in a clinical strain-specific manner. We were then prompted to evaluate the effects of EXO in *ex-vivo* patient-derived tissues. Therefore, we cultured primary nasal epithelial (HNE) cells with EXO isolated from the corresponding pwCF to mimic the native status of CF airway. Similarly, we observed a variable reduction of F508del-CFTR function in presence or absence of ETI and upregulation of proinflammatory cytokines and ROS levels. Interestingly, HNE cells treated with EXO isolated from the corresponding donor and 3 different pwCF, showed a variable reduction of ETI-dependent F508del-CFTR function mainly due to clinical strains with limited effect of patient background. Furthermore, we demonstrated that ETI pretreatment decreased the cytokines and ROS levels down to the levels of uninfected cells.
Conclusions: We observed variable ETI-rescued CFTR function, inflammatory response and oxidative stress in primary nasal epithelial cells infected with clinical exoproducts of *P. aeruginosa* strains isolated from the corresponding pwCF. These pre-clinical also studies suggest that *in-vitro* screening of patient-specific response to CFTR modulators under infection/inflammation conditions, could prove to be a valuable tool to enhance the prediction of clinical response.

This work was supported by Italian Cystic Fibrosis Research Foundation (FFC#6/2021)

1. Cantin, A.M., et al., *Oxidant stress suppresses CFTR expression.* Am J Physiol Cell Physiol, 2006. **290**(1): p. C262-70.
2. Le Gars, M., et al., *Neutrophil elastase degrades cystic fibrosis transmembrane conductance regulator via calpains and disables channel function in vitro and in vivo.* Am J Respir Crit Care Med, 2013. **187**(2): p. 170-9.
3. Cho, D.Y., et al., *LPS decreases CFTR open probability and mucociliary transport through generation of reactive oxygen species.* Redox Biol, 2021. **43**: p. 101998.

P32

Structure-guided combination of novel CFTR correctors to improve the function of F508del-CFTR in airway epithelial cells

C. Allegretta[1], D. Lunaccio[2], N. Scarano[3], V. Vinci[3], A. Salis[2], B. Tasso[3], C. Brullo[3], N. Capitanio[1], C. Piccoli[1], E. Cichero[3], E. Millo[2], O. Laselva[1]

[1]University of Foggia, Department of Clinical and Experimental Medicine, Foggia, Italy, [2]University of Genoa, Department of Experimental Medicine, Section of Biochemistry, Genoa, Italy, [3]University of Genoa, Department of Pharmacy, Section of Medicinal Chemistry, Genoa, Italy

Background: Correctors have been classified into three classes based on their mechanism of action. Type I correctors act on conformational defects between NBD1 and MSD1/MDS2, type II correctors target NBD2 and/or its interfaces and type III correctors facilitate NBD1 folding and defects caused by *F508del* [1]. The US Food and Drug Administration (FDA) and the European Medicine Agency (EMA) have approved the Vertex medicine Trikafta/Kaftrio for the treatment of Cystic Fibrosis (CF) when the disease is caused by *F508del* mutation at least one allele. Although remarkable rescue has been obtained for treatment of CF by the combination of two correctors **VX-661** (Type I corrector) + **VX-445** (type III corrector) and one potentiator **VX-770**, the stability and trafficking defects induced by the most common mutation, *F508del*, is not completely reverse [2]. Therefore, more effectively CFTR correctors are still needed.
Methods: We employed *in silico* and molecular modelling approaches to design and probe the binding site of novel series of CFTR correctors (**a-c**). The corrector activity of these novel small molecules was tested by fluorescence-based membrane potential assay (FMP) in F508del-CFTR CFBE cells and primary nasal epithelial cells.
Results: Structure-based studies allowed us to design and synthesize novel class I (**b** series) and class II (**a** series) modulators. Thus, class I modulator ability relies on interactions with Met152, Phe81, Phe191, Trp361. The design of class II corrector could be managed via NBD2-ligand H-bonds, involving Gln1261 or Val1288. Furthermore, **c** compounds have been proposed featuring putative dual corrector ability (**2c**).
Functional measurements demonstrated that eight of fourteen compounds act as CFTR corrector and the F508del-CFTR rescue was comparable to the level measured after **VX-809** or **VX-445** treatment in CFBE cells. Through rational selection based on molecular docking studies and mechanism of action, we showed that combination of compounds (**7a+1b** and **2a+2b**) targeting distinct domains of CFTR, can additively/synergistically rescue F508del-CFTR function in both CFBE cell line and primary nasal cells.
Conclusions: Our study demonstrated that in *silico* and *in vitro* approaches to develop and investigate the mechanism of action of novel CFTR correctors could be the tool to optimize the combination of correctors therapy to synergistically rescue mutated CFTR.
Acknowledgements: This study was supported by the Italian Cystic Fibrosis Foundation grant FFC#06/2021 and FFC#09/2021.
1. Okiyoneda, T., et al., *Mechanism-based corrector combination restores DeltaF508-CFTR folding and function.* Nat Chem Biol, 2013. **9**(7): p. 444-54.
2. Capurro, V., et al., *Partial Rescue of F508del-CFTR Stability and Trafficking Defects by Double Corrector Treatment.* Int J Mol Sci, 2021. **22**(10).

P33

The inverted formin INF2 in the regulation of CFTR expression and trafficking

J.F Ferreira[1], C. Alves[1], N. Pedemonte[2], V. Tomati[2], C.M Farinha[2]

[1]University of Lisboa, Faculty of Sciences, BioISI – Biosystems and Integrative Sciences Institute, Lisboa, Portugal, [2]Istituto Giannina Gaslini, U.O.C. Genetica Medica, Genova, Italy

Background: Old and new data has made increasingly clear that CFTR is at a crossing between the cytoskeleton and signaling pathways, especially cAMP signaling. Regulation of both CFTR trafficking (particularly, plasma membrane (PM) stability) and channel activation is a complex process involving protein partners functioning as molecular switches. Fine tuning of this regulation requires integrity of correct cytoskeletal organization, as the cytoskeleton is responsible for the scaffolding that stabilizes CFTR at the PM and brings several interacting proteins to CFTR's proximity, among which cAMP sensors, such as PKA and EPAC1, have a prominent role [1]. Regulation of CFTR also occurs with recruitment of several actin cytoskeleton dynamics regulators to CFTR's proximity, namely INF2. INF2 is an unusual member of the formin family because of its unique ability to accelerate actin filament depolymerization, adding to the nucleation and elongation activities common to all formins and also its unique ability to associate reversibly with the cytosolic leaflet of the endoplasmic reticulum (ER). INF2 has been reported as a negative regulator of CFTR PM stability [2].
Aims: The main goal of this work was to characterize the role of INF2 in the regulation of CFTR both at the PM and the endoplasmic reticulum (ER).
Methods: We used primary cultures of nasal epithelial cells isolated from individuals with CF, CF bronchial epithelial cells expressing wild-type or mutant CFTR and analyzed them using Western blot, cell surface biotinylation, and halide-sensitive YFP functional assay. We also used differential centrifugation to isolate the microsomal fraction in CFBE cells.
Results: Results show that
- INF2 knockdown (KD) improves rescue by Kaftrio independently of EPAC1 activation.
- EPAC1 KD and CAPZA2 KD decrease the function of Kaftrio-rescued F508del-CFTR, in agreement with previous characterization as positive CFTR regulators [1,2].
- 16HBE cells do not recapitulate the results observed in CFBE cells under INF2 KD, probably due to a reduced expression of this protein.
- INF2 expression has significant variability in nasal cells from CF, carriers and controls, whereas CAPZA2 is apparently similar.
Conclusions: Our findings show that regulation of CFTR by EPAC1, INF2 and CAPZA2 is complex, confirming the relevance of exploring their role in the crosstalk between cAMP signaling pathways and the cytoskeleton to affect CFTR modulation, and possibly CF handling.
Acknowledgements: Work was supported by FFC Ricerca grant FFC#2/2023 (to C.M.F.) and by FCT/MCTES, Portugal through UIDB/04046/2020 and UIDP/04046/2020 center grants (to BioISI) and PhD fellowship 2021.06174.BD to JFF.
References:
[1] Lobo MJ et al (2016) J Cell Sci 129, 2599–612. https://doi.org/10.1242/jcs.185629.
[2] Santos JD et al (2020) Biochem J 477, 2561–80. https://doi.org/10.1042/BCJ20200287.

P34

Novel class 3 CFTR correctors suitable for combinatorial treatments in cystic fibrosis

M. Barreca[1], F. Lo Mascolo[1], A. Lipani[1], S. Giuffrida[1], V. Spanò[1], M.V. Raimondi[1], A. Montalbano[1], P. Barraja[1], M. Renda[2], A. Borrelli[2], A. Venturini[2], D. Guidone[2], M. Genovese[2], L.J.V. Galietta[2], F. Bertozzi[3], T. Bandiera[3]

[1]University of Palermo, Department of Biological, Chemical and Pharmaceutical Sciences and Technologies (STEBICEF), Palermo, Italy, [2]Telethon Institute of Genetics and Medicine (TIGEM), Pozzuoli (NA), Italy, [3]Istituto Italiano di Tecnologia, Genova, Italy

Significant progresses have been achieved in the pharmacological treatment of cystic fibrosis (CF) with the development of Kaftrio, a triple combination of two correctors (VX-661, VX-445) and one potentiator (VX-770). However, the development of new CFTR modulators for the rescue of F508del and other CF mutations is an important goal to maximize mutant CFTR rescue.

We recently discovered a new family of CFTR correctors, named PP compounds, effective for the rescue of F508del-CFTR (1). We are presently running a multiparametric optimization of PP compounds through iterative cycles of chemical synthesis and functional evaluation. In parallel, we are also investigating another family of correctors, SH compounds, derived from PP compounds using a scaffold hopping approach. With respect to PP family, SH compounds feature a higher conformational flexibility. To assess potency and maximal efficacy, all new synthetized compounds were screened at multiple concentrations (0.1-10 μM) using the HS-YFP functional assay on CFBE41o- cells expressing F508del-CFTR mutation. So far, we have evaluated 450 compounds from which several effective compounds emerged with EC_{50} values at sub micromolar level. For the most active compounds, efficacy was also confirmed in primary airway epithelial cells derived from CF patients. Selected candidates were evaluated for *in vivo* pharmacokinetic profiling in rodents, for possible human use. Mechanistic studies have shown that PP and SH compounds act as "class 3 correctors", able to produce a strong synergistic/additive effect when combined with class 1 agents such as VX-809 and VX-661. We tested the efficacy and potency of two selected compounds, PP028 and SH157A, when combined with another type of class 1 correctors, characterized by single digit nanomolar potency (2). The combination of ARN22081, ARN22151, or ARN22361 with PP028 or SH157A markedly enhanced F508del-CFTR rescue confirming the possibility of synergism. Interestingly, the inclusion of PP028/SH157A compounds caused an increase in the potency of ARN correctors, with a shift of the EC_{50} values to the sub nanomolar range. These results confirm the ability of the PP and SH compounds to synergize with ARN correctors thus indicating the possibility to develop novel combination for optimal rescue of mutant CFTR.

1) Renda et al. Sci Rep 13:7604, 2023; 2) Pedemonte et al., Sci Adv 6:eaay9669, 2020.

Acknowledgement: development of PP and SH compounds is supported by a grant (Molecole 3.0) from the Italian Cystic Fibrosis Research Foundation (FFC ricerca). ARN correctors were also developed with the support of FFC ricerca.

P35

Primary Nasal Epithelial Cells for Personalized Medicine in non-eligible Cystic Fibrosis Patients

J. Berger[1,2], A. Balász[1,2], T. Rubil[1,2], A. Gonzáles[1], J. Berges[3,4], Y. Yun[3,4], O. Sommerburg[3,4], M. Stahl[1,2,5], M.A. Mall[1,2,5], S.Y. Graeber[1,2,5]

[1]Charité Universitätsmedizin Berlin, Department of Pediatric Respiratory Medicine, Immunology and Critical Care Medicine and Cystic Fibrosis Center, Berlin, Germany, [2]German Centre for Lung Research (DZL), Associated Partner, Berlin, Germany, [3]University of Heidelberg, Division of Pediatric Pulmonology and Allergology and Cystic Fibrosis Center, Department of Pediatrics, Heidelberg, Germany, [4]German Center for Lung Research, Department of Translational Pulmonology, Translational Lung Research Center Heidelberg, Heidelberg, Germany, [5]German Center for Child and Adolescent Health (DZKJ), partner site Berlin, Berlin, Germany

Objective: Patients with cystic fibrosis (CF) without any *F508del* allele in the cystic fibrosis transmembrane conductance regulator (*CFTR*) have currently no access to CFTR modulator therapy in Europe. In the US, the elexacaftor/tezacaftor/ivacaftor (ETI) label has been expanded to 177 rare CFTR mutations responsive in Fischer rat thyroid cells by the Food and Drug Administration (FDA). To test the potential of pharmacological rescue of CFTR by ETI in non-eligible patients with CF, we tested the *in vitro* and *in vivo* effects of ETI in non-*F508del* patients.

Methods: Highly differentiated primary human nasal epithelial cultures (pHNEC) were cultivated from nasal swabs of 39 patients with CF and non-*F508del CFTR* mutations and 29 healthy controls. 30 CF patients had non-FDA-approved mutations and 9 had FDA-approved mutations. CFTR activity was assessed by short-circuit currents in non-perfused Ussing chamber after ETI or DMSO incubation and % of healthy CFTR activity was calculated ($\Delta I_{ETI/DMSO}$%WT). In 10 patients with CF, who received ETI therapy after a preclinical response in pHNECs, we assessed lung function (FEV_1% predicted) and the *in vivo* CFTR biomarkers sweat chloride concentration (SCC) and intestinal current measurements (ICM) or nasal potential difference (NPD) at baseline and 1 to 3 months after initiation of ETI.

Results: 25 patients with CF showed no increased CFTR activity in pHNEC after treatment with ETI compared to DMSO. However, pHNECs from 14 patients with CF and non-*F508del CFTR* mutation showed a response to ETI with a mean correction of CFTR activity ($\Delta I_{ETI/DMSO}$%WT) of 20% of the healthy level ($p < 0.0001$). Among these responders, 9 patients carried FDA-approved mutations, while 5 carried non-FDA-approved variants. The 10 patients with CF who received ETI therapy showed a mean improvement in FEV_1% predicted by 12%. All 10 patients showed a pathological SCC above 60 mmol/L at baseline, which was reduced by at least 5 mmol/L after initiation of ETI ($p < 0.001$). In the ICM, the cAMP-dependent chloride secretory response increased from -12 µA/cm² at baseline to 32 µA/cm² after initiation of ETI ($p < 0.001$) and the total chloride secretory response increased from -16 µA/cm² to 123 µA/cm² ($p < 0.001$). A subgroup of 6 patients, where NPD was performed, showed an increase in total chloride response from -0.25 mV at baseline to -14 mV after initiation of ETI in the nasal epithelium.

Conclusion: We identified non-*F508del CFTR* mutations that respond to ETI *in vitro* and show clinical response and improvement in SSC, ICM and NPD. Our data show that *in vitro* CFTR modulator testing in pHNEC with confirmation of therapeutic effects with biomarkers of *in vivo* CFTR function provides a promising approach for personalized medicine in non-eligible patients with CF.

P36

Nitrate enhances the activity of F508del-CFTR channels rescued by elexacaftor-tezacaftor-ivacaftor

J.N. Charlick , D.N. Sheppard

University of Bristol, School of Physiology, Pharmacology and Neuroscience, Bristol, United Kingdom

Nitrate (NO_3^-) is a cystic fibrosis transmembrane conductance regulator (CFTR)-permeant anion, which potentiates channel gating. In particular, NO_3^- enhances the cystic fibrosis (CF)-causing, gating-deficient CFTR variants, G551D- and R117H-CFTR by increasing the frequency and duration of channel openings (1; 2). However, the action of NO_3^- on elexacaftor-tezacaftor-ivacaftor (ETI)-rescued F508del-CFTR channels is unknown. Here, we aimed to understand the effects of NO_3^- on the single-channel behaviour of ETI-rescued F508del-CFTR channels, using the patch-clamp technique.

Inside-out membrane patches were excised from Baby Hamster Kidney (BHK) cells heterologously expressing either human WT- or F508del-CFTR. F508del-CFTR expression was rescued by chronically incubating cells with elexacaftor (2 μM), tezacaftor (3 μM) and ivacaftor (1 μM) for 24 h at 37 °C. Membrane patches from WT-CFTR-expressing cells were excised into Cl^--rich intracellular solution before perfusion with a NO_3^--rich solution, while those from ETI-treated F508del-CFTR-expressing cells were maintained in either Cl^-- or NO_3^--rich intracellular solution throughout experiments. Recordings were made at 37 °C using a large [anion] gradient in the presence of 1 mM ATP. Data are means ± standard deviation (SD).

Following phosphorylation with PKA, in the presence of NO_3^-, both WT- and ETI-rescued F508del-CFTR channels displayed more frequent openings of longer duration. WT-CFTR Cl^- channels exhibited a bursting gating pattern, with both frequency and duration of openings potentiated by NO_3^-. When compared to WT-CFTR, the duration of ETI-rescued F508del-CFTR openings in the presence of Cl^- was increased, while the closed time between openings was prolonged. Notably, the frequency and duration of ETI-rescued F508del-CFTR openings were both increased greatly by NO_3^-. At -50 mV, the single-channel current amplitude (*i*) of both WT-CFTR and ETI-rescued F508del-CFTR was greatly reduced in the presence of NO_3^- (WT-CFTR: NO_3^- = -0.62 ± 0.08 pA; Cl^- = -0.75 ± 0.03 pA; n = 5; ETI-rescued F508del-CFTR: NO_3^- = -0.67 ± 0.04 pA; Cl^- = -0.74 ± 0.02 pA; n = 4). By contrast, NO_3^- markedly enhanced the open probability (P_o) of WT-CFTR and especially, ETI-rescued F508del-CFTR (WT-CFTR: NO_3^- = 0.58 ± 0.06; Cl^- = 0.39 ± 0.1; n = 5; ETI-rescued F508del-CFTR: NO_3^- = 0.44 ± 0.2; Cl^- = 0.11 ± 0.1; n = 4) to confer WT-CFTR-like levels on the variant. Finally, ETI-rescued F508del-CFTR channels exhibited similar stability at 37 °C when exposed to either Cl^- or NO_3^-.

We conclude that NO_3^- increases the P_o of ETI-rescued F508del-CFTR channels by enhancing its gating. This suggests that the potentiating effect of NO_3^- on F508del-CFTR is independent of the effects of both elexacaftor and ivacaftor, indicating that NO_3^- might help identify new CFTR potentiators.

Supported by the CF Trust.

References

1. Yeh *et al*. *J Gen Physiol*. 2015; 145:47-60.
2. Yu *et al*. *J Physiol*. 2016; 594:3227-44.

P37

CFTR expression and function in human foetal pancreatic ducts and ductal organoids

L. Kiss[1], G. Mihalekné Fűr[1], Á. Dágó[1], E.M. Orján[1], Z. Balla[1], E.R. Bálint[1], E.S. Kormányos[1], P. Pallagi[2], N. Pásztor[3], L. Kaizer[4], V. Venglovecz[5], J. Maléth[2], P. Hegyi[6,7], Z. Kozinszky[8], Z. Rakonczay Jr.[1]

[1]University of Szeged, Department of Pathophysiology, Szeged, Hungary, [2]University of Szeged, Department of Medicine, Szeged, Hungary, [3]University of Szeged, Department of Obstetrics and Gynecology, Szeged, Hungary, [4]University of Szeged, Department of Pathology, Szeged, Hungary, [5]University of Szeged, Department of Pharmacology and Pharmacotherapy, Szeged, Hungary, [6] Semmelweis University, Centre for Translational Medicine, Budapest, Hungary, [7]University of Pécs, Institute for Translational Medicine, Pécs, Hungary, [8]Blekinge Hospital, Department of Obstetrics and Gynecology, Karlskrona, Sweden

Introduction: There are multiple animal models to investigate pancreatic ductal function (i.e. bicarbonate secretion), but these have limitations due to possible differences compared to humans. However, the availability of human pancreatic tissues is largely restricted. Our goals were to characterize the structure and function of isolated foetal pancreatic ducts and organoid cultures as possible model systems. Given that CFTR has an essential role in their function and serves as a marker molecule, our study primarily focused on investigating the expression and function of the Cl^- channel in ducts and ductal organoids.

Methods: Aborted human foetal pancreata were collected at 14-23 gestational weeks (GW) (ethical permission number: SZTE-ÁOK-3415). The pancreata were utilized to isolate ducts by microdissection technique or to culture organoids. Relative gene expression levels were determined by qPCR, and protein expressions were evaluated by immunostainings. Ion transporter functions (CFTR, Na^+/H^+ exchanger [NHE], Na^+/HCO_3^- cotransporter [NBC]) were assessed via intracellular pH measurements, utilizing CFTR agonist (forskolin) or various inhibitors (CFTRinh-172, EIPA, S0859).

Results: There were no significant differences in relative gene expression levels of CFTR, NHE, NBC, SLC26A6, and the specific ductal marker cytokeratin-19 (CK-19) between tissues from 17-19 GW vs 20-23 GW. Organoid cultures also showed stable mRNA expression of ductal markers. A significant increase in CFTR protein expression was detected in tissues at 22-23GW compared to 14-15 GW. Immunohistochemical staining showed that CFTR and CK-19 expression was co-localized in human foetal pancreata from 14 GW. NHE and SLC26A6 stainings were observed from 16GW. Organoids also demonstrated expression of CFTR, SLC26A6, NHE, and CK19 proteins. Functional measurements on isolated ducts showed significantly greater recovery from alkali load at 20-23 GW than at 14-16 GW. Functional measurements on organoid cultures demonstrated CFTR, NHE and NBC activities.

Conclusions: Active ion transporters, particularly the CFTR ion channel, were already observed in isolated foetal ducts derived from 14 GW pancreata, and their activities increased as the GWs progressed. Functional organoid cultures expressing ductal marker mRNAs and proteins were successfully established. These findings provide a promising basis for utilizing human foetal pancreatic tissues for further studies of ductal bicarbonate secretion.

Acknowledgements: The study was supported by NKFIH, GINOP, EFOP (Live Longer), and MTA.

P38

Clinically approved β2 agonist formoterol enhances CFTR modulator rescue of N1303K-CFTR via a dual mode of action

M.M Ensinck[1,2], A.S. Ramalho[3], E. Bulik-Sullivan[4], M. Nijs[5], S. Van Belle[2], S. Thierie[1], L. De Keersmaecker[2], S. Munck[6], H. Klaassen[5], F. Christ[2], Z. Debyser[2], A. Hinzpeter[7], I. Sermet-Gaudelus[7], C.M. Ribeiro[4], S.H. Randell[4], F. Vermeulen[8], M.S. Carlon[1,2]

[1]KU Leuven, Chronic Diseases and Metabolism, Leuven, Belgium, [2]KU Leuven, Pharmaceutical and Pharmalogical Sciences, Leuven, Belgium, [3]KU Leuven, Development and Regeneration, Leuven, Belgium, [4]UNC-Chapel Hill, Cell Biology and Physiology, Chapel Hill, United States, [5]Cistim-CD3, Leuven, Belgium, [6]KU Leuven-VIB, Leuven, Belgium, [7]INSERM, Paris, France , [8]8UZ Leuven, Department of Pediatrics, Leuven, Belgium

Introduction: N1303K-CFTR is the fourth most common CFTR mutant and characterized by atypical molecular defects that both reduce its expression at the plasma membrane (PM) and severely impair its gating. In vitro, N1303K rescue by the CFTR modulator combination elexa-/teza-/ivacaftor (ETI) is limited. Given the distinct processing defects of N1303K compared to other CFTR mutations, we hypothesized that repurposing of CFTR correctors might not be optimal for rescuing N1303K. Therefore, we aimed to identify novel small molecules able to enhance N1303K PM expression and improve its ETI rescue.

Methods: A repurposing library of 2960 compounds was screened using an optimized high content imaging-based screening assay in A549 cells overexpressing N1303K-CFTR with an extracellular hemagglutinin tag for specific PM-CFTR labeling. Selected compounds were tested as an add-on to ETI by forskolin induced swelling in N1303K/N1303K primary rectal organoids and by Isc in primary airway cultures pre-treated w/o CF inflammatory mucopurulent sputum. Finally, their mechanism of action was investigated.

Results: From the repurposing screen, we selected 10 compounds, increasing PM expression of N1303K by 1.6-5.3 fold, to evaluate in rectal organoids. Here, the β2-agonist formoterol increased ETI rescue by ~1.5 fold, while by contrast, HDACi reduced ETI response and lowered *CFTR* expression, presumably through promoting differentiation of the stem cell-based rectal organoids. Formoterol also increased ETI rescue in differentiated primary nasal epithelia, which was enhanced when HNEs were pre-treated with CF inflammatory mucopurulent sputum. Next, we identified a dual mechanism for this compound. On the one hand, formoterol activates N1303K-CFTR ion channel function by increasing intracellular cAMP, similar to forskolin. On the other hand, formoterol increased N1303K steady-state PM expression in a dose-dependent manner without improving protein maturation. This was additive to ETI and not dependent on cAMP. Formoterol rescue of CFTR was not limited to N1303K, but was also observed for other CFTR variants, including the rare L927P mutant.

Conclusion: We identified long-acting β2-agonist formoterol, commonly used as a maintenance therapy for asthma/COPD and are currently investigating its precise mechanism. We envision this study will contribute to optimizing CFTR modulator therapies, i.e. through combinations with clinically approved repurposed small molecules like formoterol, to increase the level of CFTR rescue and linked to that clinical parameters such as FEV1. Due to its mutation-agnostic mechanism, this combination therapy can be expected to be clinically meaningful for many CFTR variants that are poorly rescued to date.

P40

Inflammation modulation using anti-inflammatory protein Tristetraprolin axed therapies

T. Dumas[1], S. Bleuse[1], K. Deletang[1], P. Boisguerin[1], A. Bourdin[1,2,3], M. Taulan-Cadars[1,2]

[1]Phymedexp, Montpellier, France, [2]University of Montpellier, Montpellier, France, [3]CHU Montpellier, Montpellier, France

Objectives: The excessive inflammatory response in CF plays a major role in lung damage. In inflammatory context some RNA-Binding protein (RNA-BP) display a deregulated expression. RNA-BPs act by binding 3'UTR extremity of mRNA, inducing their degradation or stabilization. Tristetraprolin (TTP) is an anti-inflammatory RNA-BP, that we recently reported to be down-regulated in CF cultures. TTP, when non-phosphorylated, destabilizes mRNA of pro-inflammatory proteins, like IL-6, IL-8 or TNFα, participating in resolution of inflammation. We previously identified cis and trans regulatory elements that control TTP expression and now we assess efficacy of oligonucleotides to increase the expression of TTP mRNA to modulate inflammatory response.
Materials and methods: Reporter gene vectors containing the 3'UTR regions of TTP, IL-8, IL-6 and TNFα have been constructed. The luciferase activity has been measured to evaluate the importance of different regulatory elements (miRNAs and RNA-BP). Efficacy of TTP overexpression has been evaluated by CRISPR activation (CRISPRa) assay, by expression vectors use or by an oligonucleotide-based strategy. The effect of TTP on pro-inflammatory cytokines have been evaluated by luciferase activity, mRNA and protein level quantification as well as ELISA dosage in non-CF bronchial, primary CF and THP-1 cells.
Results: Overexpression of TTP by CRISPRa or TTP expression vector, induced an increase in TTP mRNA and a reduction of pro-inflammatory cytokines mRNA level, suggesting that TTP mRNA level elevation led to inflammation resolution.
We next designed chemical modified oligonucleotides (TSB, Target Site Blocker) that prevent miRNAs and RNA-BP recruitment on the 3'UTR part of TTP mRNA. Introduction of TSB, encapsulated in cell-penetrating peptides, showed an increase in mRNA and protein TTP level in CF cells and macrophages. In inflammatory context, TTP mRNA stabilization also led to a strong decrease in pro-inflammatory mRNA in CF cells and macrophages. As well, dosage of CF and M1-derived THP-1 cells supernatants showed a decrease in pro-inflammatory cytokines secretion.
Conclusion: Identify destabilizing motifs on TTP-3'UTR led to conceive new molecular tools for inflammation resolution. Target 3'UTR sites of TTP mRNA led to stabilize mRNA and protein level associated with a cytokine mRNA and protein decrease in both CF and macrophages cells.
This work is supported by Vaincre la Mucoviscidose.
Authors declare no conflict of interest.

P41

Utilizing artificial intelligence and robotization to research the entire dynamic range of CFTR function in CF patient-derived intestinal organoids

S. Kroes , S. Spelier, B. Bosch, L. Winkel, S. Beuningen, J. Beekman

Regenerative Medicine Center Utrecht, Utrecht, Netherlands

Background: To predict clinical outcomes and treatment responses of a person with cystic fibrosis (CF), a clear CFTR-dependent read-out that measures CFTR function in a large dynamic range is essential. Multiple *in vitro* CFTR function measurement tools exist such as forskolin induced swelling (FIS) and steady-state lumen area (SLA) characterization of CF patient-derived intestinal organoids (PDIOs), which are useful for different dynamic ranges. FIS measures CFTR function within the CF domain and distinguishes CF between classes. However, FIS cannot measure CFTR function in the high-function range. This is where SLA becomes essential, as it discriminates CFTR function between CF and healthy PDIOs. A current disadvantage of SLA is that the labelling of the whole PDIO and its lumen is performed in a manual fashion. Some models have recently been developed to overcome this disadvantage, such as rectal organoid morphology analysis (ROMA). We aim to further improve these models and automate SLA by training an artificial intelligence (AI) model to differentiate between healthy and CFTR-deficient PDIOs as well as PDIOs with a broad range of CFTR function.
When combining this novel read-out with the recently described drug-induced swelling (DIS) assay and the classical FIS assay, we aim to create a single pipeline that allows for CFTR function characterization across a broad dynamic range. In order to perform this in a robust as well as high-throughput manner, we are furthermore working on developing a standardized automated workflow for the CFTR function measurements.
Altogether, the automated pipeline will allow for characterization of a broad range of CFTR genotypes. This approach will enable the diagnosis of individuals with CF who have rare or unknown mutations and, as more therapies become available in the future, support the identification of the most effective treatment for each individual.
Methods: Create an AI tool that distinguishes CFTR WT PDIOs from CFTR deficient (to different degrees) PDIOs and incorporate this in the full pipeline combining the model with DIS and FIS measurements.
Use our robot platform to automate the assay pipeline and create a standardized workflow.
Results: This project is currently in process and not yet finalized. Once we have created the overarching pipeline, assays FIS, SLA and DIS are plotted against genotypes of the CFTR2 database. The graph will show the ranges of the assays, which are currently not yet fully determined, to clarify which assay should be selected based on the % CFTR function outcomes of the different assays.
Conclusions: By automating SLA and utilizing our robot to automate our assays, we can generate more data, achieve greater standardization, and improve data validation. This approach will allow us to train a new AI model capable of creating an unbiased endpoint assay that assesses the full dynamic range of CFTR function. This pipeline will facilitate the diagnosis of individuals with CF as well as aid in identifying the most effective treatment for each individual.

P42

Mapping the Impact of ETI-mediated CFTR Reactivation On Development: a zebrafish model study

M. Cafora[1], S. Pagliughi[1], D. Dobi[1], G. Galassi[1], A. Mantero[2], M. Aureli[1], A. Pistocchi[1]

[1]University of Milan, Medical Biotechnology and Translational Medicine, Segrate, Italy, [2]IRCCS Istituto Giannina Gaslini, UOC Genetica Medica, Genova, Italy

CFTR therapy with elexacaftor/tezacaftor/ivacaftor (ETI) has transformed the life of individuals with cystic fibrosis (CF), also enhancing women's fertility. While adverse effects regarding ETI during pregnancy have not been reported, the evidence remains limited. However, it has been demonstrated that ETI can cross the placenta, therefore reaching the foetus due to placental transfer processes. The hyperactivation of CFTR through ETI administration during pregnancy could have an impact on foetus development, potentially leading to significant undesired effects. Indeed, all the functional studies conducted so far have focused on analysing the effects of CFTR loss of function/mutation, while the hyperactivation of CFTR has never been considered. In this study, we compared the effects of CFTR loss-of-function and its overexpression on zebrafish development. This model is particularly suitable for developmental studies and offers the unique opportunity to be "humanized" by injecting human genes. We compared *cftr* loss-of-function zebrafish embryos, which showed bilateral asymmetry of internal organs, increased basal inflammation levels, and delayed innate inflammatory response, with CFTR overexpressed embryos (whether in the human wild-type form or the F508del mutant form), which showed significant defects along the anterior-posterior body axis. This phenotype is reminiscent of defects in the WNT-beta signalling regulation, a pathway crucial for early developmental differentiation, such as mesoderm specification. Indeed, CFTR protein directly interacts with Dishevelled, a WNT-positive regulator, preventing it from lysosomal degradation. Importantly, this pathway can be pharmacologically modulated, opening the possibility that the undesired effects of ETI-mediated CFTR hyperactivation during development can be mitigated or avoided. In addition, since ETI has been shown to accumulate in various foetal tissues, including the developing brain, we are testing possible off-targets effects of ETI using molecular and behavioural assays during zebrafish development.
To improve the translational potential of our findings and validate the results obtained in zebrafish, we will overexpress wild-type and F508del CFTRs in human iPS and we will treat them with ETI.
This project aims to shed light on CFTR role during the first stages of life and could be essential to ensure that pregnant women with CF can safely undergo therapy with ETI.

P43

Enhancing F508del-CFTR Plasma Membrane Stability: Lipid and Modulator Synergies

D. Dobi[1], N. Loberto[1], A. Tamanini[2], R. Bassi[1], L. Mauri[1], D. Olioso[3], N. Pedemonte[4], M. Aureli[1]

[1]University of Milano, Department of Medical Biotechnology and Translational Medicine, Milano, Italy, [2]Azienda Ospedaliera Universitaria Integrata di Verona, Sezione di Patologia Molecolare, UOC Laboratorio Analisi (Sede di Borgo Trento), Dipartimento di Patologia e Diagnostica, Verona, Italy, [3]Università di Verona, Sezione di Biochimica Clinica, Dipartimento di Neuroscienze, Biomedicina e Movimento, Verona, Italy, [4]Istituto Giannina Gaslini, Genova, U.O.C. Genetica Medica, Genova, Italy

New therapeutic strategies for people with Cystic Fibrosis (CF) aim to address the defect in protein, thereby rescuing CFTR function. However, several factors contribute to the heterogeneous clinical efficacy of CFTR modulators among individuals affected by CF. The different responses could be due to alterations in the lipid compositions as a consequence of mutations impacting the trafficking of CFTR at the plasma membrane (PM) level. Indeed, our research has demonstrated that human primary bronchial epithelial cells, differentiated in vitro from individuals with CF carrying the biallelic F508del mutation, exhibit an increased content of ceramide and globotriaosylceramide at the apical membrane. Conversely, there is a decrease in the levels of cholesterol, glucosylceramide, and gangliosides, particularly GM1. This reduction in GM1 levels partly counteracts the rescue effects of elexacaftor/tezacaftor/ivacaftor (ETI) treatment, as GM1 is a crucial lipid that interacts with CFTR, promoting its stability and half-life at the apical surface. Additionally, the reduction of cholesterol content induces increased mobility of CFTR at the plasma membrane, negatively impacting the channel's stability.
Considering the important role of ganglioside GM1 and cholesterol in stabilizing CFTR at the cell surface, our aim was to investigate their beneficial effect on the maturation of F508del-CFTR rescued by ETI to optimize the effectiveness of novel F508del-CFTR modulators. Therefore, bronchial epithelial cells overexpressing F508del-CFTR were treated with ETI, with or without the exogenous administration of different molecular species of GM1 or LDL as a source of cholesterol. Exogenous administration of GM1 seems to improve the efficacy of ETI on the rescue of F508del-CFTR expression. Interestingly, the administration of molecular species of GM1 with a modification in the acyl chain reduces the destabilizing effect of VX-770 on rescued mutated-CFTR in terms of expression in cells treated with ETI, enhancing its stability at the PM. Similar results were obtained by administering LDL-cholesterol to cells.
Taken together, our data indicate that GM1, its pharmacological derivatives, and LDL-derived cholesterol are important factors that participate in the stabilization of CFTR at the PM. Based on this consideration, treatment with GM1 or its derivative and enhancing the blood concentration of LDL could be considered an innovative strategy to improve the effectiveness of the modulators on the rescue of mutated CFTR.

Acknowledgements: This work was supported by the Italian Cystic Fibrosis Research Foundation grant FFC #02_2018 FFC #02_2020, FFC #01_2022

P44

A Little Peptide for a Smooth Ride: Enhancing ETI-Mediated F508del-CFTR Stabilization with a PI3Kγ Mimetic

M. Mergiotti[1], A. Murabito[1], V. Capurro[2], A. Loffreda[3], P. Peretto[1], M. Li[1], K. Ren[1], A. Raimondi[3], C. Tacchetti[3], D. Diviani[4], N. Pedemonte[2], E. Hirsch[1,5], A. Ghigo[1,5]

[1]University of Torino, Department of Molecular Biotechnology and Health Sciences, Molecular Biotechnology Center, Torino, Italy, [2] IRCCS Istituto Giannina Gaslini, UOC Genetica Medica, Genova, Italy, [3]San Raffaele Scientific Institute Ospedale San Raffaele, Experimental Imaging Center, Milano, Italy, [4]University of Lausanne, Faculty of Biology et Medicine, Department of Biomedical Sciences, Lausanne, Switzerland, [5]Kither Biotech S.r.l., Torino, Italy

Background: Elexacaftor/Tezacaftor/Ivacaftor (ETI) therapy is the standard treatment for F508del-CFTR patients, combining two correctors (Tezacaftor [VX-661] and Elexacaftor [VX-445]) with the potentiator Ivacaftor (VX-770) to correct folding and improve gating of the mutant CFTR channel. While ETI improves lung function by 9.8% in F508del heterozygous patients after 6 months, it only partially restores CFTR channel activity and stability. Consequently, patients still face mucus dysfunction, airway infections, and inflammation, underscoring the need for enhanced treatment strategies.
Aims: Since boosting cAMP/PKA signaling promotes plasma membrane (PM) CFTR stabilization and enhances CFTR modulator efficacy, we hypothesized that the cAMP-elevating peptide we previously developed, PI3Kγ mimetic peptide (PI3Kγ MP; Patent WO/2016/103176; Ghigo et al., Sci Transl Med, 2022), could enhance F508del-CFTR PM density and stability, thereby increasing the therapeutic effect of ETI.
Methods: To assess CFTR plasma membrane (PM) density, we performed cell surface protein biotinylation followed by western blotting and immunogold electron microscopy in HEK293T, 16HBE14o-, and CFBE41o- cell lines after PI3Kγ MP treatment. Phosphoproteomics (Kinexus) was utilized to identify potential targets activated by PI3Kγ MP. Ussing chamber experiments were conducted with CF patient-derived primary human bronchial epithelial (HBE) cells to examine the stabilizing effect of PI3Kγ MP.
Results: We found that combining PI3Kγ MP with ETI maximizes ETI's therapeutic efficacy in CFBE41o- cells expressing F508del-CFTR by increasing CFTR density at PM compared to ETI alone. Notably, after 6 hours of cycloheximide (CHX) treatment, 30% of ETI-rescued F508del-CFTR remained undegraded, while 67% persisted with PI3Kγ MP treatment, effectively doubling the stability of corrected F508del-CFTR. To validate these findings, we tested PI3Kγ MP with ETI in primary human bronchial epithelial (HBE) cells, showing a 25% increase in total CFTR activity compared to ETI alone, supporting its potential as an add-on therapy for F508del CF patients. To fully unravel the underlying molecular mechanism, we performed an unbiased phosphoproteomic analysis, which identified protein kinase D1 (PKD1), a key regulator of protein trafficking, as a major target activated by PI3Kγ MP. Importantly, PKD1 inhibition blocked the PI3Kγ MP-mediated stabilization of F508del-CFTR at the plasma membrane, highlighting PKD1 as the critical mediator of this effect. Our data also suggest that PKD1 activation is driven by the coordinated actions of PKA and PKC, regulated by A-kinase anchoring protein 13 (AKAP13/AKAP-Lbc), both of which emerge as novel contributors to the CFTR signaling network.
Conclusions: This study identifies PI3Kγ and PKD1 as pivotal regulators of CFTR stability at the PM and suggests exploiting the ability of PI3Kγ MP to increase F508del-CFTR PM trafficking and ETI therapeutic effect.

This work was supported by the Italian Cystic Fibrosis Research Foundation (FFC) and the Italian Telethon Foundation.

P45

ATP-dependent modulation of the NBD1 conformational equilibrium from CFTR channel

A. Svoeglazova[1], M. Overtus[1], T. Kache[2], C. Martens[1], J. Hendrix[2], C. Govaerts[1]

[1]Universite Libre de Bruxelles, Faculty of Sciences, Biochemistry and Structural Biology unit, Brussels, Belgium, [2]Hasselt University, Advanced Optical Microscopy Centre and Biomedical Research Institute, Dynamic Bioimaging Lab, Diepenbeek, Belgium

Cystic fibrosis (CF) is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR), a chloride channel regulating fluid transport in epithelial tissues. The most common mutation, deletion of phenylalanine at the 508 position (F508del), occurs in the nucleotide-binding domain 1 (NBD1) which also contains an intrinsically disordered segment (regulatory insertion, RI) whose role is poorly understood.
We have identified an alternative conformation of NBD1, topologically different from the canonical NBD1 fold and have shown using single-molecule FRET (smFRET) microscopy, that the conformational equilibrium between canonical and alternative states is regulated by ATP (Scholl, D. et al, 2021).
This conformational role of ATP can be rationalized as the coordination of the adenine base by W401 stabilizes the canonical state. In contrast, as observed in the crystal structure of the alternative state of NBD1, W401 is unstructured, and the adenine base is not resolved.
In line with this hypothesis, we now observed using smFRET that the W401A mutation strongly promotes the alternative state.
Using differential scanning fluorimetry (DSF) we show that the stability of W401A mutant is decreased in comparison to NBD1 even in the presence of ATP.
With the help of a stabilizing nanobody, we obtained and solved the crystal structure of W401A mutant at the final 3.15 Å resolution. As predicted by our smFRET measurement, the single point mutation perturbs the canonical state, but the conformational effect is surprisingly extensive as the whole β-subdomain becomes disordered and ATP is entirely absent from its expected binding site of the domain.
We then investigated the effects of W401A mutation on full-length CFTR expressed in HEK293 cells. We observed a strong decrease in maturation and cell-surface expression of the mutant compared wt CFTR. However, HS-YFP-assays show close to normal activity, suggesting that, when present at the cell surface, the mutant is more active than the wt protein, challenging the classical model of CFTR activation.
Further studies, including single-channel activity, HDX and crystallography will be performed to investigate and decipher the molecular basis of this behaviour.

P46

Beyond chloride: a multi-omic integration analysis to identify the effects of Elexacaftor/Tezacaftor/Ivacaftor in F508del primary airway epithelial cultures

M. Kelly-Aubert[1], V. Laigle[2,3,4], E. Bardin[1,5], A. Hatton[1], C. Guerrera[6], C. Bole[7], S. Grassin Delyle[5], V. Stoven[2,3,4], I. Sermet-Gaudelus[1,8]

[1]INEM, INSERM U1151, Université Paris Cité, Paris, France, [2]Center for Computational Biology, Mines Paris-PSL, Paris, France, [3] Institut Curie, Paris, France, [4]INSERM U900, Paris, France , [5]Université Paris-Saclay, UVSQ, INSERM U1173, Montigny le Bretonneux, France, [6]Proteomics Platform 3P5-Necker, Université Paris Descartes - Structure Fédérative de Recherche Necker, INSERM US24/CNRS UMS3633, Paris, France, [7]Genomics Core Facility, Institut Imagine-Structure Fédérative de Recherche Necker, INSERM U1163 et INSERM US24/CNRS UAR3633, Université Paris Cité, Paris, France, [8]Service de Pneumologie et Allergologie Pédiatriques Centre de Référence Maladies Rares, Mucoviscidose et maladies de CFTR, Hôpital Necker Enfants Malades, Paris, France

CFTR modulator therapies such as Elexacaftor/Tezacaftor/Ivacaftor (ETI) have changed the prognosis and quality of life of CF patients, and functional studies have ascertained their role in restoring CFTR-dependent chloride secretion; however, other pathways could be improved by ETI. We undertook a multi-omic approach to decipher which pathways, other than chloride secretion, could be involved in ETI's mechanisms of action.
We generated air-liquid differentiated primary airway epithelial cultures from nasal brushings from eight F508del homozygous patients and seven healthy age- and sex-paired controls. After a 48-hour treatment with ETI or DMSO, samples were collected for transcriptomic, proteomic and untargeted metabolomic analysis.
In F508del cultures, the effect of ETI was seen at all levels with 208 significantly differentially expressed genes, 37 proteins, 16 polar metabolites and 168 apolar metabolites. Gene set enrichment analysis was performed on transcriptomic and proteomic datasets and a similar metabolite-based pathway enrichment analysis was performed on the untargeted metabolomic data. Several pathways were significantly up- or down-regulated by ETI in F508del cultures whereas no pathways were significantly modified in WT cultures.
This included the TNFa/NFKB signaling pathway, and the epithelial mesenchymal transition pathway which were down regulated by ETI, and the interferon-dependent immune response pathways, upregulated by ETI. The pathway most affected by ETI at both a transcriptomic and a proteomic level was oxidative phosphorylation. Multi-omics integration and network analysis allowed to build a network comprising the differentially expressed genes, proteins, and metabolites and highlighted key mitochondria-related pathways namely oxidative phosphorylation, Krebs cycle and fatty acid metabolism.
The convergence of our transcriptomic, proteomic and metabolomic data sets and integrated network underlines the robustness of such multi-omic approaches and pinpoints an unexpected effect of ETI on mitochondrial metabolism in F508del cells.

P47

Theranostics for People with Cystic Fibrosis: A561E-CFTR is rescued by elexacaftor/tezacaftor/ivacaftor

C.S Rodrigues, I. Pankonien, V. Railea, I. Coelho, M.D Amaral

BioISI—Biosystems & Integrative Sciences Institute, Faculty of Sciences, University of Lisboa, Lisboa, Portugal

Background: People with Cystic Fibrosis (pwCF) who carry rare or uncharacterized CFTR variants, such as p.Ala561Glu-CFTR (legacy name: A561E), are currently ineligible for highly effective CFTR modulator therapies (HEMT). However, previous studies have suggested that some rare CFTR variants may still respond to HEMT. The forskolin-induced swelling (FIS) assay, using patient-derived organoids, is a very promising tool to evaluate rare CFTR variants responses to HEMT.
Objective: To predict the clinical responses to HEMT of pwCF carrying the p.Ala561Glu-CFTR variant through tests in patient-derived intestinal organoids.
Methodology: Intestinal organoids were generated from 3 pwCF, two carrying p.Ala561Glu-CFTR variant in homozygosity and one in heterozygosity with p.Gly542Ter (legacy name: G542X). CFTR function was assessed through the forskolin induced swelling (FIS) microscopy assay. CFTR modulators tested included: potentiator VX-770 (ivacaftor, iva) alone or in combination with correctors VX-661(tezacaftor, teza) and/or VX-445 (elexacaftor, elexa).
Results: Our results show that p.Ala561Glu-CFTR is rescued by the approved triple CFTR modulators combination, elexa/teza/iva to the clinical benefit threshold (i.e., response of pPhe508del/pPhe508del organoids to elexa/teza/iva), even when the second allele is a class I CFTR variant [52% of the response observed of pPhe508del/pPhe508del organoids to elexa/teza/iva]. These data are in line with our previous data obtained in primary cultures of human bronchialepithelial cells [1] Clinical data before and after treatment for this patients is currently being assessed to evaluate the HEMT benefit on pwCF carrying the p.Ala561Glu-CFTR variant and their correlation with FIS values.
Conclusion: This work highlights the importance of testing approved HEMT in rare variants and the relevance of using intestinal organoids as a tool for personalized medicine/theranostics to predict the responsiveness to CFTR modulators for pwCF carrying rare CFTR variants who are thus ineligible to undergo classical clinical trials.
***Acknowledgements**: Work supported by UID/04046/2025 centre grant (to BioISI), from FCT/MCTES Portugal, and research grants "HIT-CF" (H2020-SC1-2017–755021) from EU; CR and VR are recipients of PhD fellowships SFRH/BD/153053/2022 from BioSys PhD programme PD/00065/2012 and SFRH/BD/06718/2021, respectively. and IP of contract DOI 10.54499/DL57/2016/CP1479/CT0013, all from FCT (Portugal).*
[1] Awatade et al (2014) EBioMedicine vol. 2,2 147-53

P48

Proteases overactivation in predisposing the cystic fibrosis airway epithelium to infections

P. Bigot[1], M. Badaoui[1], R. Vivès[2], M. Chanson[1]

[1]University of Geneva, Departement of Cell Physiology and Metabolism, Geneva, Switzerland, [2]University of Grenoble, Institute of Structural Biology, Grenoble, France

Structural changes in the CF lungs result from a protease/anti-protease imbalance associated with tissue degradation and loss of pulmonary homeostasis favouring recurrent respiratory infections by opportunistic bacteria such as *Pseudomonas aeruginosa* (*Pa*). We have recently shown that the CF airway epithelium exhibits a defect in apicobasal polarization, characterized by luminal deposition of fibronectin that promotes the adhesion of *Pa* to the epithelial surface (1). Interestingly, it is not fibronectin in its integral form that is responsible for *Pa* adhesion, but the fragments generated by proteolysis of the protein. To target efficiently *Pa* adhesion, it is crucial to uncover the proteases involved in fibronectin remodelling. We did not observe quantitative changes in proteases secretion by CFTR knockdown (CFTR-KD) airway epithelial Calu-3 cells as compared to control (CFTR-CTL) cells. Using a fluorogenic substrate, however, a tremendous increase in matrix metalloproteases (MMPs) activity was measured in the CF cell secretome. MMPs are secreted as inactive pro-forms and further activated in a tightly regulated manner in the extracellular matrix. Using chemicals that activate pro-MMPs into active MMPs, we found that the increased MMPs activity in the CFTR-KD secretome was due to an over-activation of MMPs. Among MMPs, MMP-7, which is critical during the wound repair process, is known to degrade fibronectin. We confirmed that MMP-7 is over-activated in the CFTR-KD secretome whereas its expression was not different from control secretome. Moreover, specific pharmacological inhibition of MMP-7 blunt most of MMPs' activity detected in the CFTR-KD secretome. Finally, inhibition of MMP-7 prevented fibronectin remodelling. Mechanistically, glycosaminoglycans such as chondroitin sulfate (CS) have been shown to modulate MMP-7 activity. In this context addition of chondroitinases to the CFTR-KD secretome reduced fibronectin remodeling, suggesting that CS may play a role in promoting MMP-7 activity. These results indicate that suppression of the MMP-7 overactivity may represent a potential mean to prevent *Pa* adhesion to the CF airway epithelium.

1. Badaoui, M., *et al.*, *Vav3 Mediates Pseudomonas aeruginosa Adhesion to the Cystic Fibrosis Airway Epithelium.* Cell Reports, 2020. **32**(1): p. 107842.

P49

Iterative *in silico* and *in vitro* screens identify compounds simultaneously improving CFTR channel function and biogenesis

M.-C. Ardelean[1], S. Javor[2], J.-L. Reymond[2], P. Vergani[1]

[1]University College London, Neuroscience, Physiology and Pharmacology, London, United Kingdom, [2]University of Bern, Chemistry, Biochemistry and Pharmaceutical Sciences, Bern, Switzerland

Objective: Cystic fibrosis (CF) is caused by mutations in the *CFTR* gene, encoding a Type IV ATP-binding cassette (ABC) transporter uniquely functioning as an anion channel. The current standard-of-care combination treatment, ETI (Elexacaftor-Tezacaftor-Ivacaftor, Vertex Pharmaceuticals), has significantly improved patient outcomes. However, there remains substantial potential for discovering new, cost-effective CF treatments. Expanding upon our earlier findings, we modified our screen to test both CFTR activity and biogenesis, aiming to identify molecules with the potential of repairing both defects in mutant variants.

Methods: In a first screen, we had evaluated the acute (3 min exposure) effect on wild-type CFTR (WT-CFTR) activity of 109 candidate molecules extracted from a database of 4.9 million compounds. The computational workflow combined both molecular docking and ligand-based *in silico* screening. The compounds were selected based on the structural attributes of G-compounds[1,2], initially designed as inhibitors for the bacterial ABC transporter MsbA (also Type IV). One compound (Hit1) emerged from this first screen as a promising hit. Upon further testing, it was found to significantly enhance both biogenesis and channel function of both WT-CFTR and F508del-CFTR under chronic conditions (24 h incubation).

Building on these findings, we performed a second ligand-based virtual screen of the original 4.9 million compound database to identify 30 structural neighbours to Hit1. A total of 139 compounds (the original 109 and the additional 30) were subsequently tested under chronic conditions to characterize simultaneously CFTR membrane proximity and channel function. An image-based fluorescence assay[3] was employed to monitor CFTR biogenesis (quantified as membrane proximity, ρ) and anion flux (Area Above the quenching Curve, AAC7) in HEK293 cells, exploiting the co-expression of iodide-sensitive YFP (H148Q/I152L)[4] fused to CFTR, and cytosolic mCherry for precise image segmentation.

Results and Conclusions: Top hit compounds improving CFTR membrane proximity and/or channel function compared to Hit1 are shown in Table 1. Future research will focus on evaluating the effects of these molecules on F508del-CFTR, both independently and in combination with ETI. These results underscore the potential for discovery of more effective CFTR modulators, simultaneously repairing both biogenesis and gating defects.

Table 1: Effects of selected compounds on WT-CFTR channel function and membrane proximity.

Test Conditions (acute treatment includes 0.1 µM forskolin)	**AAC7** Mean (95% CI)	**Log(ρ)** Mean (95% CI)	**n** number of plates
DMSO (chronic)	0.77 (0.68, 0.87)	-0.15 (-0.17, -0.13)	12
+ 1 µM I (acute) 3 µM E + 5 µM T + 1 µM I (chronic)	2.44 (2.34, 2.53)	0.04 (-0.02, 0.10)	12
+ 10 µM Hit1 (acute) 10 µM Hit1 (chronic)	1.76 (1.53, 1.99)	-0.10 (-0.13, -0.06)	8
+ 10 µM Hit1.1 (acute) 10 µM Hit1.1 (chronic)	1.91 (1.77, 2.05)	-0.08 (-0.13, -0.03)	8
+ 10 µM Hit1.2 (acute) 10 µM Hit1.2 (chronic)	1.90 (1.71, 2.08)	-0.14 (-0.16, -0.11)	4
+ 10 µM Hit1.3 (acute) 10 µM Hit1.3 (chronic)	0.95 (0.69, 1.22)	-0.04 (-0.10, 0.02)	4

References:

1. Ho, H. et al. Nature 557, 196–201 (2018)
2. de Jonge, H. R. et al. FEBS Lett 594, 4085–4108 (2020)
3. Prins, S. et al. J Biol Chem 295, 16529–16544 (2020)
4. Galietta, L.J. et al. FEBS Lett 499, 220-224 (2001)

P50

CFTR activity in nasal epithelia from subjects with different genotypes

V. Capurro[1], E. Pesce[1], V. Tomati[1], C. Pastorino[1], M. Lena[1], A. Dighero[1], L.J.V. Galietta[2,3], F. Cresta[4], C. Castellani[4], N. Pedemonte[1]

[1]IRCCS Istituto Giannina Gaslini, UOC Genetica Medica, Genoa, Italy, [2]Telethon Institute of Genetics and Medicine (TIGEM), Pozzuoli, Italy, [3]University of Naples Federico II, Department of Translational Medical Sciences (DISMET), Naples, Italy, [4]IRCCS Istituto Giannina Gaslini, UOSD Centro Fibrosi Cistica, Genoa, Italy

The human nasal epithelial (hNE) cells are an interesting *ex-vivo* model to study the molecular and functional activity of the CFTR protein. To assess the CFTR activity in people with cystic fibrosis (pwCF) and its rescue by modulators, reference values are necessary, that may be resembled by CFTR activity levels observed in subjects not affected by CF.
Limited data have been collected so far showing the range of activity of normal CFTR.
In the frame of a project aimed to characterize rare CFTR mutations, we collected hNE cells (by nasal brushing) from a cohort of about forty non-CF donors. We generated well-differentiated nasal epithelia in air-liquid interface, and then we performed short-circuit current measurements by Ussing chamber to evaluate CFTR-dependent Cl- secretion. We opted for two different experimental conditions: first, we performed the measurements using symmetrical Cl- solution (without applying a Cl- gradient; standard condition) and then by imposing a Cl- gradient (gradient condition). This latter condition allowed us to maximize the anion transport by CFTR, by minimizing the contribution of other factors that might limit the driving force for CFTR-mediated Cl- secretion.
Our data showed that, under standard condition, CFTR activities measured in our cohort varied between 10 and 35 $\mu A/cm^2$. In the majority of subjects, when we switched to the gradient condition, we observed a two-fold increase in CFTR activity. However, two small groups of subjects had different behaviours. Interestingly, both groups were composed by cultures showing the lowest levels of CFTR activity (ranging from 10 to < 20 $\mu A/cm^2$) in standard condition. However, in gradient condition, the first group showed a higher improvement, around 3-fold, in CFTR activity. The second group showed a limited increase, around 1.5-fold, with CFTR-mediated currents that were lower than 30 $\mu A/cm^2$.
Our cohort included not only healthy subjects but also obligate carriers and interestingly we found that all of them were indeed inside the second group, together with some “healthy” donors. Intrigued by this, we analysed the CFTR mRNA of the nasal epithelia derived from “healthy” donors and we found that all of them were indeed carriers of CFTR variants that are reported to impair, at least partially, CFTR activity (although not necessarily CF-causing). On the contrary, no variants were detected in other samples (not belong to this second group) tested in parallel. Finally, we analysed the effect of gradient condition on epithelia derived from pwCF and found that, under this condition, no increase in CFTR activity was detected as compared to standard condition.
These results support the thesis of the robustness of the nasal model as a predictive model. In fact, it allowed us to detect subclinical CFTR dysfunction (as that of obligate carriers), and to unmask some previously unidentified carriers present in the cohort.

Supported by Fondazione Ricerca Fibrosi Cistica grants FFC #9/2019 and FFC #10/2021, Italian Ministry of Health grants GR-2018–12367126 and PNRR-MR1-2023-12378412 and Cystic Fibrosis Foundation grant PEDEMO20G0.

P51

Optimizing scalable nasal airway organoid generation for automated high-throughput screening

A. Shekhar[1], L. Winkel[1], S. Beuningen[1,2], G.Amatngalim[1], J. Beekman[1,2]

[1]Wilhelmina Children's Hospital, Regenerative Medicine Center Utrecht, University Medical Center, Pediatric Pulmonology, Utrecht, Netherlands, [2]Centre for Living Technologies, Utrecht, Netherlands

Introduction: Organoids have revolutionized CF research by enabling personalized CFTR drug response assays. However, current organoid generation and assay methods are labor-intensive, limiting throughput to low or medium levels. To address these limitations, we aim to optimize airway organoid generation and image-based screening for greater scalability and faster workflows, facilitating high-throughput CFTR function assays.
Methods: Airway basal cells from nasal brushings of pwCF (N=2) were expanded in 2D cultures and differentiated using two approaches: (1) ALI cultures on transwell inserts and (2) submerged differentiation in thermoreactive dishes, offering higher scalability and faster culture times. Organoids, derived from epithelial fragments of these cultures and maintained for >4 weeks, were automatically dispensed into 384-well plates using an OT-2 liquid handler. Optimization focused on BME concentrations and dispensing parameters to achieve uniform organoid density.
Results: Both ALI and submerged differentiation methods successfully generated airway organoids, which maintained stable spheroid structures and motile cilia at the apical surface for over a month. Automated dispensing ensured consistent organoid distribution in 384-well plates while preserving phenotype and viability.
Conclusion: The submerged differentiation method enables scalable organoid generation, and preliminary tests confirm the feasibility of automated organoid dispensing into 384-well screening plates. These results demonstrate the potential for integrating airway organoids into automated high-throughput CFTR screening workflows.

P52

Rescue of p.Phe508del-CFTR through inhibition of the kinase GRK5: identification of proteins and small molecule mediators

M.F. Caleiro[1], D. Neugebauer[1], S. Abdul[1], I. Michou[1], R. Pacheco[2,3], M.D. Amaral[1], H.M. Botelho[1]

[1]BioISI – Biosystems & Integrative Sciences Institute, Faculty of Sciences, University of Lisboa, Lisboa, Portugal, [2]Instituto Superior de Engenharia de Lisboa (ISEL), Departamento de Engenharia Química, Lisboa, Portugal, [3]Centro de Química Estrutural—Institute of Molecular Sciences, Universidade de Lisboa, Lisboa, Portugal

Introduction: CFTR modulators have revolutionized the pharmacological management of Cystic fibrosis (CF), leading to considerable improvements in quality of life for people with CF (pwCF) who are eligible. At the molecular level, restoration of p.Phe508del-CFTR activity requires addressing its folding, traffic, stability and gating defects, whose standard of care is the Highly Effective Modulator Therapy (HEMT) comprising correctors tezacaftor/VX-661 and elexacaftor/VX-445, and potentiator ivacaftor/VX-770. Nevertheless, these drugs do not fully restore clinical efficacy endpoints of pwCF to carrier levels, suggesting that therapeutic improvements may still be achievable by more potent molecules or additional combination therapies. Our group identified G protein-coupled receptor kinase 5 (GRK5) as a novel p.Phe508del-CFTR regulator and CF drug target: GRK5 inhibition restores p.Phe508del-CFTR trafficking to the PM and activity in cell lines and primary bronchial epithelial cells in a way additive to modulators [1]. Targeting GRK5 might therefore contribute to improve CF pharmacotherapy.
Objective: to elucidate the mechanism of the signalling pathway coupling GRK5 inhibition to functional p.Phe508del-CFTR rescue in cellular models.
Methods: CFBE cells expressing a fluorescent CFTR traffic reporter (mCherry-Flag-p.Phe508del-CFTR [2]) were used to screen a library of 419 siRNAs targeting 212 genes previously identified by our team as CFTR traffic regulators [2]. This loss-of-function screen was performed on cells treated with either specific GRK5 inhibitors (CCG-273463, CCG-273441) or vehicle. To determine how these genes relate to the endoplasmic reticulum quality control (ERQC) checkpoints we rescreened the same siRNA panel using cells expressing p.Phe508del-CFTR genetic revertants [3,4]. Small molecules associated with p.Phe508del-CFTR rescue were identified by LC-MS-based metabolomics.
Results: Microscopy analysis revealed 19 genes whose expression was required for p.Phe508del-CFTR traffic restoration by CCG-273463. The involvement of these genes in the GRK5-CFTR signalling pathway is being validated by rescreening under the more potent CCG-273441 GRK5 inhibitor, applied in combination with VX-661 and VX-445. An analysis of the metabolomic profile of cells with GRK5 under basal or inhibited conditions is underway, highlighting metabolites – and, potentially, enzymatic activities – involved in CFTR functional rescue.
Conclusion: Our data explore an uncharacterized signalling pathway that maximizes rescue of p.Phe508del-CFTR by VX-661/445 through inhibition of GRK5. The overall relevance of our data and implication for potential improvement in CF pharmacological management will be discussed.
Acknowledgements: *Work supported by UID/04046/2025 centre grant (to BioISI) from FCT, Portugal, and research grants NewKinCF 2022.03453.PTDC (DOI: 10.54499/2022.03453.PTDC, FCT, Portugal) (to HMB) and ORGESTRA Doctoral Network (HORIZON-MSCA-2022-DN-01-01-101120108) from EU (to MDA).*

[1] Botelho *et al* (2025) submitted.
[2] Botelho *et al* (2015) *Sci Rep* **5**:9038.
[3] Roxo-Rosa *et al* (2006) *PNAS USA* **103**: 17891-6.
[4] Farinha *et al* (2013) *Chem Biol* **20**: 943-55.

P53

Study of potential therapeutic approaches for cystic fibrosis patients with rare mutations in Sri Lanka

T. Gunawardena[1], S. Kay[1], G. Langeveld[1], A. K.W.D. Anuradha[2,3], E. Jasinghe[3], F. Ratjen[4,5], M. Solomon[5], V. Dissanayake[2], C. E. Bear[1,6,7]

[1]Research Institute, The Hospital for Sick Children, Molecular Medicine, Toronto, Canada, [2]University of Colombo, Faculty of Medicine, Colombo, Sri Lanka, [3]Lady Ridgeway Hospital, Colombo, Sri Lanka, [4]Research Institute, The Hospital for Sick Children, Translational Medicine, Toronto, Canada, [5]The Hospital for Sick Children, Division of Respiratory Medicine, Toronto, Canada, [6] University of Toronto, Department of Physiology, Toronto, Canada, [7]University of Toronto, Department of Biochemistry, Toronto, Canada

For many years Cystic Fibrosis (CF) was considered as a rare genetic disease present among the Caucasian population. However, potentially there are tens of thousands individuals that remain undiagnosed, with a large percentage in low and middle incomes countries (LMIC)(1). The potentially large number of CF cases in the Asian region are believed to be under reported due to the absence of newborn screening programs, limited diagnostic tools, and lack of disease awareness among both health care providers and the general population. There are likely novel CF -causing variants in these regions as well. In Sri Lanka, in addition to the common variant, F508del- a number of rare mutations such as C1282C>G, Y913C, V456A, 1161del C, 185+1G>T, 2184insA and CFTRdel4-11 have been identified (2). Currently there are approximately 29 paediatric patients who are diagnosed with CF in the country on the basis of high sweat chloride measurements and CF-associated clinical signs, several harbouring variants that have the potential to benefit from Trikafta through a philanthropic program developed by Vertex. The molecular defects of certain variants are not well understood and hence, the in-vitro response to Trikafta is not yet tested. In the current study we have evaluated the consequences of Trikafta treated in vitro on the rare missense mutations identified in Sri Lanka.

Our experiments were conducted in HEK293 cells transiently transfected with WT or V456A-CFTR constructs. The WT constructs were pre-treated with DMSO, while the V456A-CFTR constructs were pre-treated with DMSO or Elexacaftor (E) and Tezacaftor (T) for 48hrs. The fluorescence membrane potential (FMP) studies were carried out for both WT and V456A mutants by acute treatment of Forskolin and Ivacaftor. The potentiation of DMSO treated V456A transfected HEK cells showed an activation of 1.17±0.06 ΔF/F0 while the pretreatment with ET led to ivacaftor-potentiated CFTR channel activation to 1.61±0.17 ΔF/F0 above baseline. The rescue effect of Ivacaftor was 37% of the mean response of WT-HEK cells while the rescue effect of ETI combination was 95% of the mean response to the WT-HEKs cells.

Based on previous literature, even though we expected V456A to have residual function and therefore respond to Ivacaftor (3) we only saw a modest response for Ivacaftor treated V456A-CFTR HEK constructs while Trikafta seems to have a better rescue effect on this mutation.

Acknowledgements: The authors would like to thank the Structural and Biophysical core facility at the Hospital for Sick Children, Toronto, ON, Canada, for providing the facility to conduct FMP assays. We also acknowledge funding by Cystic Fibrosis Canada and Cystic Fibrosis Foundation, US.

References:

1. Guo JK, I.; Hill, A. International disparities in diagnosis and treatment access for cystic fibrosis. Pediatric Pulmonology. 2024;59:1622–30.
2. Indika NLR, Vidanapathirana DM, Dilanthi HW, Kularatnam GAM, Chandrasiri N, Jasinge E. Phenotypic spectrum and genetic heterogeneity of cystic fibrosis in Sri Lanka. BMC Med Genet. 2019;20(1):89.
3. Preston E. Bratcher, Kenneth C. Hunt, Kyle Pickard, Jennifer L. Taylor-Cousar. Positive clinical response to ivacaftor treatment in an individual with the CFTR genotype F508del/V456A, Journal of Cystic Fibrosis. 2019; 18(2)

P54

A novel active conformation of CFTR may drive functional recovery of F508del-mutant

C. Govaerts[1], M. Overtus[1], T. Rubil[2], B. Loughlin[3], Z. Rich[3], M. Rodrat[4,5], Z. Yang[6], A. Balazs[2], J.C. Kappes[6], M. Mall[2], D.N. Sheppard[4], J.F. Hunt[3]

[1]Université Libre de Bruxelles, Biochemistry and Structural Biology, Brussels, Belgium, [2]Charité-Universitätmedizin Berlin, Pneumologie, Immunologie and Intensivmedizin, Berlin, Germany, [3]Columbia University, Department of Biological Sciences, New York, United States, [4]University of Bristol, Pharmacology and Neurosciences, Bristol, United Kingdom, [5]Mahidol University, Institute of Molecular Biosciences, Nakhon Pathom, Thailand, [6]University of Alabama School of Medicine, Heersink School of Medicine, Birmingham, United States

The F508del mutation in Cystic Fibrosis Transmembrane Conductance Regulator (CFTR), the leading cause of Cystic Fibrosis (CF), disrupts protein folding and stability, severely impairing chloride channel function. Despite advancements in treatment with the modulators elexacaftor, tezacaftor, and ivacaftor (ETI), current therapies fail to fully restore CFTR activity. In this study, we explored the structural and functional mechanisms underlying CFTR activity by combining ETI with the CFTR-stabilizing nanobody T2a, delivered via lipid nanoparticles (LNPs). Co-expression of T2a, which thermally stabilizes the nucleotide-binding domain 1 (NBD1), enhances mutant CFTR expression, maturation, and activity.

Functional assays demonstrated that the combination of T2a and ETI not only restores mutant CFTR activity but also stabilizes channel function over prolonged periods, overcoming the thermal instability that limits ETI efficacy. Cryo-electron microscopy revealed two distinct T2a-bound CFTR conformations: an inactive "V-shaped" state, where T2a prevents canonical NBD1-NBD2 dimerization, and a novel active state characterized by NBD1 detachment.

This latter conformation reorganizes the transmembrane helices into a dynamic, pore-like structure compatible with ion conduction. Notably, binding of T2a promotes the emergence of subconductance states, rarely observed in wild-type CFTR but prominent in F508del-CFTR. These states, which represent partially open channel configurations, are likely intermediates facilitated by NBD1 detachment that enable dynamic reorganization of the CFTR transmembrane region.

These findings redefine our understanding of CFTR gating and channel function, particularly for mutant CFTR, and underscore the therapeutic potential of stabilizing nanobodies to enhance both structural integrity and functional output in protein trafficking diseases.

P55

Synergy between stabilizing nanobody and approved correctors enables remarkable functional recovery of F508del-CFTR

M. Overtus[1], T. Rubil[2], J.N. Charlick[3], M. Rodrat[3,4], A. Balázs[2], M. Mall[2], D.N. Sheppard[3], C. Govaerts[1]

[1]Université Libre de Bruxelles, Biochemistry & Structural Biology, Brussels, Belgium, [2]Charité – Universitätsmedizin Berlin, Klinik für Pädiatrie m.S. Pneumologie, Immunologie und Intensivmedizin, Berlin, Germany, [3]University of Bristol, School of Physiology, Pharmacology and Neurosciences, Bristol, United Kingdom, [4]Mahidol University, Center for Advanced Therapeutics, Institute of Molecular Biosciences, Nakhon Pathom, Thailand

Cystic Fibrosis (CF) is caused by mutations in the Cystic Fibrosis Transmembrane conductance Regulator (CFTR) chloride channel, with F508del being the most common. Current treatments, including elexacaftor, tezacaftor, and ivacaftor (ETI), partially restore CFTR function but fail to achieve full recovery, leaving significant therapeutic gaps. In this study, we developed a lipid nanoparticle (LNP)-based system to deliver mRNA encoding the CFTR-stabilizing nanobody T2a, designed to thermally stabilize the nucleotide-binding domain 1 (NBD1). This innovative approach allows intracellular nanobody expression to complement existing correctors.

Our results demonstrate a remarkable synergy between T2a and ETI, resulting in enhanced F508del-CFTR trafficking and maturation, as evidenced by a significant increase in fully glycosylated CFTR and cell-surface expression. Functional assays confirmed that this synergistic improvement translates into substantial recovery of chloride channel activity, approaching near-normal levels in some contexts. The LNP formulation ensures efficient delivery and expression in relevant cell types, providing a scalable, mRNA-based therapeutic platform.

This work highlights the potential of nanobody-based molecular chaperones to address the limitations of existing CF therapies, opening pathways for personalized and enhanced treatments for people with CF and other protein trafficking disorders.

P57

Esc peptides enhance CFTR gating and combat *Pseudomonas aeruginosa*

M.L. Mangoni[1], L. Ferrera[2], M. Mori[3]

[1]Sapienza University of Rome, Biochemical Sciences, Rome, Italy, [2]Istituto Giannina Gaslini,, Genova, Italy, [3]University of Siena, Siena, Italy

Background: Cystic fibrosis (CF) is a genetic disorder caused by mutations in the CFTR gene, which encodes a chloride channel crucial for airway function. While existing modulators have improved CFTR activity in many cases, persistent lung infections, particularly from *Pseudomonas aeruginosa*, remain a significant challenge for some patients. Recent studies have revealed a novel function of Esc peptides: their ability to serve as CFTR potentiators (1). These peptides effectively enhance the function of F508del CFTR, a mutation that impairs protein folding, trafficking, and gating.
Objectives: The study aimed to evaluate the ability of Esc peptides and their analogs to act as dual-function molecules, potentiating activity of defective CFTR and exhibiting antimicrobial activity against *Pseudomonas aeruginosa* in cystic fibrosis (CF)-like conditions.
Methods: Electrophysiology experiments and computational studies were conducted to assess the effect of Esc peptides and de-novo designed analogs on CFTR gating mutations. Antimicrobial activity was tested under conditions mimicking CF pulmonary ion transport and mucus obstruction.
Results: In this work, electrophysiology and computational analyses have demonstrated that Esc peptides and their engineered analogs can rescue the function of defective CFTR mutants, likely upon direct interaction with CFTR's nucleotide-binding domains. The most promising peptide for the dual antimicrobial and CFTR potentiator activities was also investigated for its activity against *Pseudomonas aeruginosa* under CF-like conditions of ion transport and mucus obstruction and found to cause 2 log10 reduction in the number of viable bacterial cells in the lungs
These findings open new avenues for the development of innovative CF therapies that integrate CFTR potentiation with broad-spectrum antibacterial effects, addressing both the underlying genetic defect and secondary infections in CF patients.
Conclusions: Esc peptides represent promising candidates for developing novel therapies targeting CF lung disease, integrating CFTR potentiation with broad-spectrum antibacterial activity.

1. **Ferrera L**, Cappiello F, Loffredo MR, Puglisi E, Casciaro B, Botta B, Galietta LJV, Mori M, Mangoni ML.Cell Mol Life Sci. 2021 Dec 31;79(1):67. doi: 10.1007/s00018-021-04030-2.

This work was supported by the Italian Cystic Fibrosis Foundation (Project FFC#4/2022) Delegazione FFC Ricerca di Roma e della Franciacorta e Val Camonica (to M.L.M).

P58

Unveiling new insights of ionocytes' role in cystic fibrosis using single-cell RNA sequencing analysis and human induced pluripotent stem cell-derived airway epithelial cells

M. Salvà-Barceló[1,2], J. Muncunill-Farreny[2], L. Vallier[3,4], J.M. Ramis[1,2,5], M. Monjo[1,2,5], M. Vilà-González[1,2,5]

[1]University of the Balearic Islands, Group of Cell Therapy and Tissue Engineering (TERCIT) - Research Institute on Health Sciences (IUNICS), Palma de Mallorca, Spain, [2]Health Research Institute of the Balearic Islands (IdISBa), Palma de Mallorca, Spain, [3]Berlin Institute of Health (BIH) at Charité, BIH Center for Regenerative Therapies, Berlin, Germany, [4]Max Planck Institute for Molecular Genetics, Berlin, Germany, [5]University of the Balearic Islands, Departament of Fundamental Biology and Health Sciences, Palma de Mallorca, Spain

Pulmonary ionocytes are a rare airway epithelial cell (AEC) type expressing high levels of the cystic fibrosis transmembrane conductance regulator (CFTR). CFTR is an ion transport channel whose loss of function mutations are the underlying cause of cystic fibrosis (CF) disease. Thus, ionocytes have been hypothesised to play a key role in the pathophysiology of CF.

Even though single-cell RNA sequencing (scRNA-seq) technology has enabled the detailed transcriptomic profiling of AECs, including ionocytes, the function and disease-specific transcriptomic changes of these cells are still unclear. To address this question, we have explored publicly available scRNA-seq data from air-liquid interface (ALI) cultures derived from primary bronchial epithelial cells aiming to identify transcriptomic disease-specific changes between ionocyte populations from healthy individuals (control or CO) and from CF patients (Carraro *et al.*, 2021). Our analysis has revealed more than 10 genes that are differentially expressed in CF vs CO ionocytes. Additionally, gene set enrichment analysis has indicated transcriptomic changes related to biological processes such as regulation of immune response, oxidative phosphorylation and cilia movement.

However, these findings are based on transcriptomic data of samples with different genetic backgrounds and require to be validated using isogenic *in vitro* models. The study of ionocytes is restricted due to the limited availability of primary tissue and the lack of appropriate models. Human induced pluripotent stem cells (hiPSC) could be a promising approach as they can be expanded indefinitely and can be differentiated providing an unlimited source of AECs. Moreover, hiPSCs can be easily genetically modified to correct CFTR mutations to generate isogenic controls from patient-derived cells. Nevertheless, their differentiation into AECs can be challenging as there is a lack of standardized protocols and most of them have failed to consistently produce rare AECs such as ionocytes. In previous studies we have successfully developed an *in vitro* airway model using hiPSC lines from CF patients and isogenic counterparts (wildtype or WT) that offers a platform to study ionocytes. Isogenic CF and WT lines were parallelly differentiated using our previously described protocol (Vilà-González *et al.*, 2024) to generate an isogenic hiPSC-AEC *in vitro* model to validate our transcriptomic findings. After 28 days of AEC maturation in ALI culture, airway epithelium was characterised through different molecular biology techniques including immunofluorescent staining. We have confirmed the presence of abundant AEC types (basal, goblet and secretory cells) along with less frequent cell types such as ionocytes and pulmonary neuroendocrine cells (PNECs), which show characteristic morphology. Moreover, we have confirmed the expression of key genes of interest from our transcriptomic analysis in our isogenic *in vitro* model.

Our hiPSC-AEC isogenic *in vitro* model contains both abundant and rare AECs and has allowed us to validate some of our transcriptomic findings. This model also has the potential to be used to study other aspects of CF and other respiratory diseases. Furthermore, our findings provide novel insights into the potential role of ionocytes in CF pathophysiology, bringing us a step closer to identifying new therapeutic approaches.

P59

GY971, a new anti-inflammatory agent for the treatment of Cystic Fibrosis lung disease

I. Lampronti[1,2], C. Tupini[1], S. Fagnani[1], G. Raso[1], A. Chilin[3], G. Marzaro[3], A. Tamanini[4], N. Pedemonte[5], V. Capurro[5], O. Laselva[6], C. Allegretta[6], A. Bragonzi[7], G. Cabrini[1,2]

[1]University of Ferrara, Department of Life Sciences and Biotechnology, Ferrara, Italy, [2]University of Ferrara, Center of Innovative Therapies for Cystic Fibrosis (InnThera4CF), Ferrara, Italy, [3]University of Padova, Department of Pharmaceutical and Pharmacological Sciences, Padova, Italy, [4]University of Verona, Department of Engineering for Innovation Medicine, Verona, Italy, [5]IRCCS Istituto Giannina Gaslini, U.O.C. Medical Genetics, Genova, Italy, [6]University of Foggia, Department of Clinical and Experimental Medicine, Foggia, Italy, [7]IRCCS San Raffaele Scientific Institute, Infections and Cystic Fibrosis Unit, Milan, Italy

In *Cystic Fibrosis* (CF), chronic lung inflammation and progressive pulmonary damage persist despite the use of *highly effective CFTR modulator therapy* (HEMT). For this reason, there is an urgent need for innovative anti-inflammatory agents to be administered via the pulmonary route to people with CF (pwCF), working through novel mechanisms and maintaining an acceptable side effect profile. GY971 is a new synthetic furocoumarin derivative developed to inhibit NF-kB/DNA complexes and characterized by anti-inflammatory action, at nanomolar concentrations, based on the regulation of neutrophil chemotaxis mediators in CF bronchial epithelial cells *in vitro* and murine lungs *in vivo*. The study was focused on the analysis of GY971 in CF bronchial epithelial cell lines exposed to *P.aeruginosa* (PAO-1) or TNF-a *in vitro*, and in mouse models of *P.aeruginosa* lung infection *in vivo*. The anti-inflammatory effect of GY971 was also validated in primary HBE (Human Bronchial Epithelial) cells and HNE (Human Nasale Epithelial) cells derived from pwCF.
Thanks to the promising results obtained, GY971 was recently approved by the *European Medicines Agency* (EMA) as orphan drug for CF. Ongoing research aims to consolidate its efficacy and safety profile, bringing it closer to clinical application as an innovative anti-inflammatory treatment for CF lung disease.

P60

Hyaluronic acid derivates as new potential anti-inflammatory agents for Cystic Fibrosis lung disease

I. Lampronti[1,2], S. Fagnani[1], C. Tupini[1], G. Raso[1], G. Cabrini[1,2], S. Manfredini[1,2], A. Baldisserotto[1]

[1]University of Ferrara, Department of Life Sciences and Biotechnology, Ferrara, Italy, [2]University of Ferrara, Center of Innovative Therapies for Cystic Fibrosis (InnThera4CF), Ferrara, Italy

In people with cystic fibrosis (pwCF), increased chronic inflammation is one of the most important outcomes. Although highly effective CFTR modulator therapy (HEMT) benefit pwCF, and the well-known anti-inflammatory drug ibuprofen provides longer survival, lung inflammation persists, resulting in a progressive lung damage. Considering the limited effect on lung inflammatory process obtained even with the most effective CFTR modulators, and the percentage of pwCF that could not benefit from ETI, the need of anti-inflammatory drugs as long-term pulmonary therapy is clearly recommended even in the era of HEMT.
Therefore, one of the possible therapeutic approaches is based on the regulation of the recruitment of neutrophils inside CF airway lumen, targeting pro-inflammatory molecules, including the main neutrophil chemokine Interleukin 8 (IL-8), known biomarker of CF lung inflammation. In this field new hyaluronic acid (HA) derivatives cross-linked with amino acids have been synthesized and analyzed; different derivatives seem to reduce the expression of neutrophil chemotaxis mediators in CF bronchial epithelial cells. One of the new HA derivates displayed also antimicrobial activity against *Pseudomonas aeruginosa*, thus showing the potential to be used for multiple aspects. In conclusion, this research aims to select the most promising HA derivates to be utilized as possible anti-inflammatory treatment in the CF lung disease to improve the quality of life of people affected by this chronic condition.

P61

Mitochondrial metabolic rewiring drives wound healing in cystic fibrosis primary airway epithelium cultures treated with Elexacaftor/Tezacaftor/Ivacaftor

M. Kelly-Aubert[1], E. Bardin[1,2], V. Laigle[3,4,5], A. Deslys[1], A. Hatton[1], B. Chevalier[1], I. Kühl[6], A. Imbard[7], E. Le Guillou[1,7], P. Vargas[1], V. Stoven[3,4,5], I. Sermet-Gaudelus[1,8]

[1]INEM, INSERM U1151, Université Paris Cité, Paris, France, [2]Université Paris-Saclay, UVSQ, INSERM U1173, Montigny le Bretonneux, France, [3]Center for Computational Biology, Mines Paris-PSL, Paris, France, [4]INSERM U900, Paris, France, [5]Institut Curie, Paris, France, [6]Institute of Integrative Biology of the Cell, UMR9198, CEA, CNRS, Université Paris-Saclay, Gif-sur-Yvette, France, [7]Department of Biochemistry, Assistance Publique-Hôpitaux de Paris, Paris, France, [8]Service de Pneumologie et Allergologie Pédiatriques Centre de Référence Maladies Rares, Mucoviscidose et maladies de CFTR, Hôpital Necker Enfants Malades, Paris, France

CFTR modulators have changed the prognosis and quality of life of patients, but their exact mechanism of action remains incompletely understood. A multi-omic study in primary nasal epithelial cells from F508del children highlighted mitochondria-related pathways as being an important target of Elexacaftor/Tezacaftor/Ivacaftor (ETI). The present study analyzes the impact of ETI on several key mitochondrial pathways in F508del primary cultures. ETI induced an increase in mitochondrial mass as shown by a significant increase in both TOMM20 immunostaining and mitochondrial DNA to nuclear DNA ratio. This was concomitant with a significant increase in *PGC1a* and *TFAM* mRNA expression, key regulators of mitochondrial biogenesis. Mitochondrial activity assessed by oxygen consumption rates (OCR) in Seahorse assays revealed that ETI restored deficient maximal OCR and spare respiratory capacity in F508del cultures to WT levels.
We next assessed fatty acid oxidation flux in F508del cultures by administering deuterated palmitate and quantifying the downstream fatty acids. This highlighted an increase in fatty acid oxidation flux upon ETI as well as a significant increase in the expression of the fatty acid oxidation rate-limiting enzyme CPT1. As fatty acid metabolism is essential for airway epithelium repair[1] , we performed wound healing assays and demonstrated that F508del cells have a lower wound healing rate than WT cells and that this is restored with ETI. The addition of the CPT1 inhibitor, Etomoxir, inhibited fatty acid oxidation and abolished the effect of ETI on wound healing rates. This suggests that ETI induces mitochondrial metabolic rewiring involving oxidative phosphorylation and fatty acid oxidation and that this participates in enhanced airway epithelium repair capacities highlighting a new role of CFTR in airway epithelial repair and metabolic pathways.

1. Crotta, S., Villa, M., Major, J. et al. Repair of airway epithelia requires metabolic rewiring towards fatty acid oxidation. Nat Commun 14, 721 (2023)

P62

Evaluation of amniotic epithelial cells (hAECs) as a cell-based therapeutic approach for cystic fibrosis

M. Lena[1,2], V. Tomati[1], V. Capurro[1], E. Pesce[1], C. Pastorino[1], A. Dighero[1], R. Gramignoli[3], N. Pedemonte[1]

[1]IRCCS Istituto Giannina Gaslini, UOC Genetica Medica, Genova, Italy, [2]University of Genoa, Department of Neurosciences, Rehabilitation, Ophthalmology, Genetics, Maternal and Child Health (DINOGMI), Genova, Italy, [3]IRCCS Istituto Giannina Gaslini, UOSD Laboratorio Terapie Cellulari, Genova, Italy

Background: Cystic fibrosis (CF) is a genetic multiorgan disease due to mutations that cause the loss of function of the cystic fibrosis transmembrane conductance regulator (CFTR) protein, that acts as a chloride/bicarbonate channel. Loss-of-function mutations occurring in the CFTR channel lead to decreased chloride secretion, paralleled by increased sodium absorption, impairing mucociliary clearance in the airways.
In recent years human amniotic epithelial cells (hAECs), derived from placental tissue, have gained considerable attention in the field of regenerative medicine due to their potential. hAECs have unique characteristics such as an excellent proliferative and differentiation capacity, as well as being easily available and safe. Many studies have proven that their use can be useful in different fields such as: neurological, pulmonary, cardiovascular and hepatic diseases.
Aim: The aim of our study is to investigate whether the excellent differentiating capacities of hAECs could be exploited for a possible cell-based therapy, capable of generating a differentiated airway epithelium expressing a mature and functional CFTR protein.
Methods: As a first evaluation, we co-cultured (under different conditions and cell ratios) hAECs with primary human bronchial epithelial (HBE) cells derived from a CF patient compound heterozygous for F508del and a minimal function, not-rescuable mutation. The differentiation process was carried out on snapwell supports and under air-liquid interface conditions for 4 weeks. Subsequently, electrophysiological studies were performed in Ussing chamber to verify the presence of CFTR activity.
Results: hAECs co-cultured with HBEs did not compromise HBEs viability or differentiating capability. Our preliminary results suggest that hAECs might promote proliferation of non-differentiated cells and improve epithelial differentiation. Interestingly, when we treated “co-cultured” epithelia with CFTR modulators, we observed a higher CFTR activity than that measured in epithelia composed by HBE cells alone. Further investigation will be needed to understand the mechanisms by which hAECs improve these parameters. Although very preliminary, these results suggest that hAECs may provide strong paracrine support for the innate regenerative capacity of lung tissue.

Supported by the Italian Ministry of Health

P63

Modeling a Triple Culture Airway-on-a-Chip for Inflammation Studies in Cystic Fibrosis

Y. Man[1,2], A. Jiang[1,2], A. Stejskalová[1], H. Bai[3], G. Di Bonaventura[4], M. Romano[4], D. Ingber[1,2,5], R. Plebani[4]

[1]Wyss Institute for Biologically Inspired Engineering - Harvard University, Boston, United States, [2]Boston Children's Hospital - Harvard University, Boston, United States, [3]Xellar Biosystems, Boston, United States, [4]"G. d'Annunzio" University of Chieti-Pescara, Department of Medical, Oral and Biotechnological Sciences, Chieti, Italy, [5]Harvard John A. Paulson School of Engineering and Applied Sciences - Harvard University, Cambridge, United States

Background: Cystic fibrosis (CF) is characterized by chronic lung inflammation and uncontrolled polymorphonuclear leukocyte (PMN) migration. We developed the first CF Airway-on-a-Chip model, composed of CF human bronchial epithelial cells (HBEs) and non-CF human vascular endothelial cells (HVECs), demonstrating increased inflammation and PMN migration compared to a non-CF chip. However, it is clear that HVECs and fibroblasts play a relevant role in CF airway inflammation. Thus, an airway chip built with all CF cellular components will be fully representative of a CF airway unit, offering a high-fidelity human CF preclinical model suitable for disease modeling and pharmacological studies.
Aims: Aim of this study was to develop the first CF triple culture Airway-on-a-Chip prototype in a microfluidic device composed of CF-HBEs, CF-HVECs, and CF stroma (CF Airway-on-a-Chip 2.0).
Methods: CF and non-CF HBEs, HVECs and fibroblasts were cultured in a two-channel microfluidic chip. Cells were characterized by immunocytochemistry and confocal imaging. Cytokine levels were measured by Luminex® and analyzed with the Prism GraphPad software.
Results: We established the first CF triple culture in a microfluidic device with differentiated epithelium containing ciliated, goblet, club, and basal cells in the upper channel, and fibroblasts and endothelial cells in the basal channel. Preliminary experiment comparing CF Airway-on-a-Chip 2.0 to a control chip with non-CF cells revealed increased PMN recruitment, with the endothelium playing a central role. The CF culture uniquely secreted detectable P-selectin and exhibited higher TNF-α levels than the non-CF counterpart. Moreover, CF-HVECs cultured alone released higher ICAM-1, TNF-α, IL-8, IL-6, GM-CSF, and MIP-1α levels compared to non-CF HVECs, alongside a decrease in MCP-1 levels. This correlated with increased PMN adhesion.
Conclusions: The CF Airway-on-a-Chip 2.0 provides an implemented version of the previous model, enabling the study of additional CF disease features as stromal remodeling and endothelial dysfunction. It highlights the pivotal role of endothelial cells in CF inflammation, especially in neutrophil recruitment, thus offering a valuable platform for exploring new treatments targeting CF inflammation.

P64

Gut microbiota-metabolome relationships across pwCF taking highly effective CFTR modulators: Preliminary findings from the GRAMPUS-CF study

R. Marsh[1], W. Cheung[1], D. Arends[1], D. Sills[2,3], D.W Rivett[4], A.R Smyth[2,3,5], C. van der Gast

[1]Northumbria University, Department of Applied Sciences, Newcastle upon Tyne, United Kingdom, [2]University of Nottingham, Academic Unit of Lifespan & Population Health, School of Medicine, Nottingham, United Kingdom, [3]NIHR Nottingham Biomedical Research Centre, Nottingham, United Kingdom, [4]Manchester Metropolitan University, Department of Natural Sciences, Manchester, United Kingdom, [5]Queen's University Belfast, School of Medicine, Dentistry and Biomedical Sciences, Belfast, United Kingdom

Background: Relationships between gut microbiome structure, intestinal physiology, and wider gastrointestinal symptoms are yet to be fully elucidated within cystic fibrosis. Despite shifts to highly effective CFTR modulators, people with cystic fibrosis (pwCF) still experience negative intestinal symptoms and exhibit signs of a general gut microbiota dysbiosis as compared to controls from the wider population. Further understanding of disease interplay is therefore warranted at the intestinal site, particularly to support the potential development of additional interventions, therapeutics, and clinical practices to relieve morbidity across pwCF.
Aims: Baseline faecal samples across children and adults from the Gut Research Advancing a Mechanistic and Personalised Understanding of Symptoms in Cystic Fibrosis (GRAMPUS-CF) study were utilised to investigate relationships between microbiota composition and the wider intestinal metabolome.
Methods: Gut microbiota profile was determined using PacBio full-length 16S HiFi sequencing (n = 70). Untargeted metabolomics, including additional lipidomics, were performed with ultra-high-performance liquid chromatography high-resolution mass spectrometry (UHPLC-HRMS) (n = 64). Correlation-based approaches were used to construct networks highlighting relationships between taxa and metabolites across paired samples Participant clinical data was further integrated to investigate potential associations with microbiota structure and function under treatment with elexacaftor/tezacaftor/ivacaftor.
Results: From our preliminary analyses, we confidently identified a wide array of unique metabolites from the metabolomic (*n* = 660) and lipidomic approaches (*n* = 527). Network analyses highlighted both positive and negative associations across beneficial and potentially pathogenic taxa, extending to relationships with metabolites and lipids of physiological interest in the CF intestine. pwCF clinical demographics also influenced the microbiota, metabolome and lipidome.
Conclusions: Faecal multi-omics indicate important relationships with gut microbiota structure across pwCF undertaking CFTR modulator therapy. Temporal dynamics of the intestinal microbiome will be investigated across elexacaftor/tezacaftor/ivacaftor (ETI) therapy in the GRAMPUS-CF cohort. Further clinical data and intestinal physiology metrics will be available to aid our analyses. This also includes the integration of participant intestinal symptom data.
Acknowledgements: Many thanks to the Cystic Fibrosis Trust for funding this work (SRC 023). Most importantly we would like to thank all the participants have enrolled onto GRAMPUS-CF. Without them this research would not be possible.

P65

The gut's role in pulmonary and extrapulmonary manifestation of Cystic Fibrosis

C. Cigana[1], G. Delmonte[1], A. Facoetti[2], L. Veschetti[1], F. Gianferro[1], A. Neri[1], R. Fiorotto[3], A. Livraghi-Butrico[4], L. Massimino[2], F. Ungaro[2], A. Bragonzi[1]

[1]San Raffaele Scientific Institute, Division of Immunology, Transplantation, and Infectious Diseases, Milano, Italy, [2]San Raffaele Scientific Institute, Division of Immunology, Transplantation, and Infectious Diseases, Experimental Gastroenterology, Milano, Italy, [3] Yale School of Medicine, Department of Internal Medicine, Section of Digestive Diseases, Yale Liver Center, New Haven, United States, [4]The University of North Carolina, Cystic Fibrosis/Pulmonary Research and Treatment Center, Chapel Hill, United States

Introduction: Cystic Fibrosis (CF) research primarily focuses on the lung, limiting understanding of disease pathogenesis and chronic infections in pwCF, including those on CFTR-modulator treatment. This gap may result from insufficient studies on distal organs.
Objectives: We developed a new mouse model of CF with deltaF508-Cftr mutation into genetically diverse Collaborative Cross (CC) background (CC037 HO). The aim was to have a suitable genetic background for CF, uncover novel pathological mechanisms, and explore the role of distal organs beyond the lung.
Methods: Microbiological and inflammatory responses were characterized by standard techniques (bacterial culture, FACS, and immunostaining), as well as genomic tools (metatranscriptomics and single-nucleus RNA-seq).
Results: Early-life respiratory inflammatory profiles in the lung and blood were evident in CC037 HO mice. Sn-RNA-seq showed upregulated immune response pathways, along with enhanced defense responses to bacteria. Microbiological cultures showed enteric bacteria, with *Escherichia coli*, *Enterococcus faecalis*, and *Klebsiella oxytoca* being the most prevalent in the lung of CC037 HO but absent in WT mice. Metatranscriptomics revealed shared microbiota between the colons and lungs of CC037 HO mice, whereas CC037 WT mice showed distinct microbiota. Similar enteric bacteria were found in the livers of CC037 HO mice, where inflammation was detected. Gut of CC037 HO mice was characterized by mucus accumulation, inflammatory responses, and a higher bacterial load in mucosa and stool compared to WT mice. This was associated with compromised barrier integrity and increased gut permeability, enabling potential organ access. Treatment with laxative rescued gut barrier integrity, and respiratory and systemic inflammation in CC037 HO mice.
Conclusion: Our data support the role of the gut in systemic effects, with potential impacts on distal organs such as the lungs and liver.

Supported by Italian CF Research Foundation and CF Foundation.

P67

Development of a novel Adenine Base Editor with temporospatial control of the editing

I. Zollo[1], L. Santos[1,2,3], J. Alves[1,4], A.S Coroadinha[2,3], P.T Harrison[4,5], C.M Farinha[1]

[1]BioISI - Biosystems and Integrative Sciences Institute, Faculty of Sciences, University of Lisboa, Lisbon, Portugal, [2]iBET, Instituto de Biologia Experimental e Tecnológica, Oeiras, Portugal, [3]Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras, Portugal, [4]Physiology Department, University College Cork, Cork, Ireland, [5]Cincinnati Children's Hospital, Cincinnati, OH, United States

Despite the great advances witnessed with the approval of CFTR modulators, 10-15% of people with cystic fibrosis (pwCF) still lack a causal treatment, namely those with nonsense variants that abolish protein synthesis. In these cases, gene-based therapies represent the best possibility for a cure.
Among all genome editing techniques available, Adenine Base Editor (ABE) is one of the most promising as it uses a Cas9 nickase (nCas9) fused to a TadA enzyme that can convert any adenine (A) in the editing window into guanine (G), being therefore suitable for the correction of W1282X mutation, the second most common CF-causing variant lacking a therapy.
Besides the development of an effective gene editing tool, it is not clear yet which cell types in the pseudostratified lung epithelium (and how many of them) need to be edited to restore CFTR function to levels that correspond to clinical benefit.

To find an answer to these fundamental questions, we developed a basal cell line bearing the nonsense W1282X mutation (BCi W1282X-CFTR) that retains the ability to differentiate into all the cell types of the airway epithelium and a new CRISPR-based tool, which is a split version of ABE (SplitABE), that allows temporal control of the editing. Our preliminary results show that SplitABE is able to reverse the premature stop codon (TGA) back to the WT (TGG) and the temporal control of the editing reduces the levels of bystander edits, usually associated with ABE[1].
Here, we now report a strategy to couple the temporal regulation of the ABE with spatial control to allow us to tackle this question. To do this, we cloned the SplitABE cassette under the control of five different cell-type specific promoters, covering the main cell types in the airway epithelium (basal, ciliated, secretory type 1 and 2, ionocytes)[2].

The five final plasmids obtained were used to transfect the packaging cell line 293T to produce lentiviral particles on a large-scale. To determine lentiviruses infectious titers, the BCi W1282X-CFTR cell line was transduced with different dilutions of the viruses and the DNA was extracted after 72h. The absolute amount of target DNA (viral gene) was quantified by Droplet Digital PCR (ddPCR).
Taking into consideration the number of transduced cells, the volume and the dilution of the viruses, we calculated an LV titer between 7E+06 and 1,5E+07 IU/mL for the different viruses.
According to these quantifications, we are currently transducing the BCi W1282X-CFTR cells with the LV at various MOIs to generate novel cell lines with cell type-specific expression of the SplitABE.

In conclusion, this novel gene editing tool combined with the newly generated cell model will allow us to identify the cellular targets for CFTR correction and optimize the system for a possible transition into the clinic.

1. Santos L et al (2023) J Cyst Fibr 22 (S2), S32.
2. Zollo I et al (2024) J Cyst Fibr 23 (S2), S162.
Acknowledgements: Work supported by grants HARRIS21G0 and FARINH24G0 from CFF and centre grants UIDP/04046/2020 and UIDB/04046/2020 (doi: 10.54499/UIDB/04046/2020) from FCT, Portugal (to BioISI).

P68

Correction of F508del via Interhomolog Homologous Recombination in compound heterozygote cell lines

L. Lonetti[1], J.B. Murray[1], C.M. Lee[2], P.T. Harrison[1,3]

[1]University College Cork, College of Medicine and Health, Department of Physiology, Cork, Ireland, [2]University College Cork, School of Biochemistry and Cell Biology, Cork, Ireland, [3]Cincinnati Children's Hospital, Division of Pulmonary Medicine, Cincinnati, United States

Since its discovery in 2013, CRISPR technology has been under constant advancement and its employment as a therapeutic approach to Cystic Fibrosis (CF) is ever-growing. The appeal of this technology is mostly due to the incredible specificity of the system, and, in principle, it has the potential to correct many cystic fibrosis transmembrane conductance regulator (CFTR) mutations by introduction of insertions, deletions, or base substitutions in the genome. However, not all variant categories are as easy to address, with disease severity influenced by mutation class, modifier genes, and the presence of complex alleles characterised by multiple mutations on the same allele.

Of the over 650 known to be disease-causing sequence variants of the CFTR gene that have been identified, ~51% of people with CF have compound heterozygous genotypes; of these, 82% present at least one F508del allele. The approach described here exploits multiple nicks induced by Cas9 nickase to trigger interhomolog homologous recombination to correct F508del in compound heterozygous cell lines. Specifically, the correction is driven by a primary nicking guide tailored to the mutation, paired with a secondary nicking guide positioned at a distance; the latter being established through indel testing of possible suitable sgRNAs. As this approach does not require the induction of double-stranded breaks, it significantly lowers the rate of unwanted insertion/deletion events (indels) without sacrificing editing efficiency. Indeed, this is confirmed by our preliminary Next Generation Sequencing results in the IB3-1/S9 F508del/W1282X cell line – showing an increase of the wild type allele from 32.68% to 33.15% and a corresponding decrease of the F508del allele from 67.31% to 66.85% – and in the 16HBEge F508del/WT cell line – showing an increase of the wild type allele from 41.47% to 47.46% and a corresponding decrease of the F508del allele from 58.53% to 52.54% – along with a negligible percentage of indels (0.047%).

Our next steps are to increase the editing efficiency through optimisation of the nicking guides and transfection methods, carry out a long-range PCR to further verify the allele-specific editing, and assess protein recovery and functional rescue, ultimately moving on from F508del to tackle some of the rarer disease-causing variants.

P69

Precise correction of challenging mutations causing cystic fibrosis through transient delivery of the prime editing machinery

M. Bulcaen[1], L.L. Dipalo[1,2], B. Poelmans[2], S. Thierie[2], M. Smits[1], M. De Roy[2], M. Ensinck[2], A. Ramalho[3], Z. Debyser[1], F. Vermeulen[3], R. Gijsbers[1], M. Carlon[2]

[1]KU Leuven, ADVANTAGE: Advanced Disease Modelling, Targeted Drug Discovery and Gene Therapy, Leuven, Belgium, [2]KU Leuven, Department of Chronic Diseases and Metabolism, Leuven, Belgium, [3]KU Leuven, Department of Development and Regeneration, Leuven, Belgium

Introduction: In the last decade, highly effective CFTR modulator therapy has revolutionized the treatment of cystic fibrosis (CF). However, the most severe CF-causing variants – namely, indel and nonsense mutations – remain completely refractory to modulators, leaving ~10% of the individuals with CF without viable treatment options. A promising therapeutic approach for these challenging mutations is gene editing – which, through a one-time administration, could lead to their targeted and durable correction in the genome of clinically relevant cell types. While several CF-causing nonsense mutations are amenable to base editing, indels can only be corrected by prime editing (PE) owing to the unique capability of the latter to introduce any type of point mutation in the genome. The clinical translation of prime editing would be greatly expedited by a "hit-and-run" delivery system, which could guarantee the desired on-target gene correction while minimizing the risk of off-target edits that is associated with long-term expression of the gene editing machinery. Virus-like particles (VLPs), which transiently deliver the gene editing machinery in the form of a ribonucleoprotein, are ideal candidates for this purpose.
Methods and results: Here, we leveraged PE to tackle W1282X (c.3846G>A) and c.3528del – the fifth and 23rd most common CF-causing *CFTR* variants, respectively. While W1282X could theoretically be corrected though base editing, we found that this strategy resulted in the introduction of deleterious bystander edits.
We tested several prime editing approaches in HEK293T cells expressing the mutant *CFTR*-cDNA, and identified optimal strategies that precisely corrected W1282X and c.3528del with an efficiency of 8 ± 1% and 40 ± 2 %, respectively. The editing restored similar percentages of CFTR ion channel function, which was measured through halide-sensitive YFP quenching. We then validated the best editing strategies in patient-derived intestinal organoids – a highly clinically relevant model. Transduction of the organoids with lentiviral vectors encoding the components of the prime editing machinery resulted in the rescue of CFTR function, which we assessed by measuring forskolin-induced organoid swelling. In parallel, we focused on the improvement of VLPs as delivery vehicles for PE RNPs. Through the refinement of culture and transfection conditions of producer cells, as well as VLP architecture and purification strategies, we were able to achieve a ~10-fold increase in the efficiency of VLP-mediated PE in a fluorescence-based HEK293T reporter system.
Conclusions: Our results showcase prime editing as a uniquely versatile tool that can be harnessed to precisely and permanently correct challenging CF-causing mutations like W1282X and c.3528delC. Efforts to further optimize VLPs with the aim of achieving robust VLP-mediated PE in organoids are currently ongoing.

P70

Whole Exon Correction Approach for CF: Repair of All CF-causing Variants in Exon 12 with Homology-Directed Repair

E. Collins[1], J. Murray[1], J. Alves[1,2], P. Harrison[1,3]

[1]University College Cork, Department of Physiology, Cork, Ireland, [2]University of Lisboa, BioISI-Biosystems and Integrative Sciences Institute, Faculty of Sciences, Lisboa, Portugal, [3]Cincinnati Children's Hospital, Division of Pulmonary Medicine, Cincinnati, United States

Precision gene editing using CRISPR/Cas9 is a promising therapeutic approach to treat genetic disorders, such as cystic fibrosis (CF). Double-strand breaks (DSBs) induced by the Cas9/gRNA are repaired by end-joining mechanisms, which are imprecise and frequently result in nucleotide insertions or deletions (indels), or by homology-directed repair (HDR). HDR uses endogenous or exogenous DNA as a template to mediate precise repair and has been applied to efficiently correct individual CF-causing variants *in vitro*. However, given >700 CF-causing variants have been described, approaches which correct more than one variant are needed. CF-causing variants are frequently found clustered within exons, such as *CFTR* exon 12 which harbours 18 variants, making whole exon correction approaches particularly beneficial.

Application of HDR in this regard is hindered by its short editing window and the high incidence of indel byproducts. The decline in HDR editing efficiency with increasing distance from the DSB is well-described; with collapse of the D-loop structure formed between target and template DNA reported to be responsible. Here, we hypothesise the high sequence identity typically shared between the target DNA and HDR template (HDRT) repair tract triggers early D-loop collapse, limiting HDR. As such, we propose incorporation of silent edits within the repair tract will reduce sequence identity, prevent D-loop collapse, and extend the editing window. Through testing of this hypothesis, we present a single HDRT-based strategy to repair all CF-causing variants in exon 12.

In order to track HDR extension across exon 12 at the DNA and protein level, by amplicon sequencing and western blot respectively, a 5' (c.1585-1G>A), middle (G542X) and 3' exon 12 variant (c.1679+1G>A) were studied. Three cell lines, each bearing one of the variants of interest, were created in CFTR expression minigenes stably expressed in HEK293 cells. The gRNA was targeted to intron 12, 13bp downstream of c.1679+1G>A. HDRTs with reduced target identity were generated by incorporation of base substitutions within the repair tract, which spanned from 5' exon 12 to the DSB. As indel byproducts occur alongside HDR, two end-joining inhibitors, AZD7648 and ART558, were also tested.

Using a HDRT with a repair tract possessing 100% target identity (HDRT100), HDR efficiency declined with increasing distance from the DSB, being highest in c.1679+1G>A cells (7.1±1.5%), followed by G542X (4.7±1.9), and c.1585-1G>A (0.6±0.4%). When a repair tract with 70% target identity was used (HDRT70), HDR was generally found to be higher yet a decline in efficiency was still observed in comparison of c.1585-1G>A (2.1±1.8%) with G542X (8.5±4.2%) and c.1679+1G>A cells (7±3.2%). Strikingly, when end-joining inhibitors were employed HDR efficiency drastically increased in all cell lines with all HDRTs (n=4). Under these conditions no HDRT which can wholly extend HDR was identified; HDR was lowest in c.1585-1G>A (15.9±15% HDRT100, 12.7±2% HDRT70) and highest in c.1679+1G>A cells (20.7±14% HDRT100, 28.1±5% HDRT70). Although further work is required to establish potential benefits of reducing HDRT target identity, thus far, we have robustly demonstrated a single HDRT can repair three exon 12 CF-causing variants.

P72

Humanized CFTR exon-replacement mice to accelerate development of nucleic acid-directed therapies

R. Conlon, C. Hodges

Case Western Reserve University, Genetics and Genome Sciences, Cleveland, United States

While small molecule drugs are effective therapies in people with cystic fibrosis (CF) who have alleles that make protein, therapeutic approaches acting on mutant DNA or RNA are needed for patients with alleles that do not. Because nucleic acid-directed therapeutics are sequence-specific and mouse and human CFTR gene sequences differ, we sought to generate mice with not just the disease mutation but also with local sequence context. Such mice could allow testing of the same therapeutic which would be used in people with CF. Because the human and mouse CFTR genes have 27 exons with matching exon-intron boundaries, we were able to use an exon-replacement strategy. Individual mouse Cftr exons and short segments of flanking introns were replaced with their human cognates by CRISPR/Cas gene editing in mice. We tested if mouse Cftr alleles with wild-type human exon-replacements are fully functional by replacing exons 3, 11, 12, 23 or 26. We found that all 5 homozygous wild-type human exon-replacement strains are wild type in phenotype with full CFTR channel activity. We generated exon-replacements with CF mutations: E60X in exon 3, F508del in exon 11, G542X and R553X in exon 12, W1282X in exon 23, and Q1411X in exon 26. All 6 mutant exon-replacements have CF phenotypes as homozygotes, with phenotypic severity and channel activity matching expectations from mouse and human mutations. We restored function of the mutant exon-replacement genes with candidate therapeutic approaches in vitro, demonstrating that the strains are valid models for testing therapeutics. Finally, we corrected the R553X mutation in mice with a base editor delivered to airway basal stem cells by lipid nanoparticles, showing that the exon-replacement strains are powerful models in vivo. We conclude that exon-replacements can accelerate the development of nucleic-acid directed therapeutics for cystic fibrosis. The exon-replacement strains of mice are distributed to the international cystic fibrosis research community by the Cystic Fibrosis Mouse Resource Center (cfmice.org).

P73

A split and inducible precise adenine base editor for a less promiscuous correction of W1282X

J. Alves[1,2,3], L. Santos[4,5], C. M Farinha[2], P. T Harrison[3]

[1]Biosciences Institute, University College of Cork, Department of Physiology, Cork, Ireland, [2]BioISI-Biosystems and Integrative Sciences Institute, Faculty of Sciences, University of Lisbon, Lisbon, Portugal, [3]Division of Pulmonary Medicine, Cincinnati Children's Hospital, Cincinnati, OH, United States, [4]iBET, Instituto de Biologia Experimental e Tecnológica, Oeiras, Portugal, [5]Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras, Portugal

Cystic Fibrosis (CF) nonsense mutations account for roughly 23% of disease-causing variants. Approximately 6 out of 7 people with CF who have a nonsense mutation on one allele will, fortunately, be compound heterozygous with a modulator-responsive variant on the other allele, but that leaves 1 in 7 people who will have a second variant that does not respond to modulators.

W1282X, the fifth most common CF-causing variant, generates a premature terminating codon (PTC), leading to a lack of full-length functional CFTR protein production.

Adenine base editing (ABE) can potentially correct any CF-nonsense point mutation at a guide RNA-programmed target site, without generating double-stranded breaks, thus leading to a safer therapeutic approach than a traditional CRISPR/Cas9 strategy, due to the minimal levels of unwanted insertions/deletions. Typically, ABE uses a fusion of a Cas9 nickase and a synthetic TadA enzyme modified to convert any Adenine (A) into Guanine (G) in an editing window covering varying stretches of nucleotides in the spacer region, dependent on the promiscuity of the deaminase.

The Harrison lab has previously reported efficient A>G editing of W1282X[1]. However, high levels of bystander-effect were observed, leading to the unwanted generation of the R1283G variant. Based on previous reports of reduced off-target/bystander-effect and duration of nuclease activity upon splitting one of the proteins of the Cas-9-TadA complex from ABE7.10-SpRY into two halves, we designed two fusion proteins, NLS-FKBP12-Cas9(C)-NLS and TadA-NES-Cas9(N)-FRB, that should dimerize and edit in the presence of rapamycin; we refer to this two proteins collectively as splitABE.

The parental ABE7.10-SpRY and splitABE editing window was characterised in four different genomic loci in HEK293T cells to assess editing capacity and reduction in bystander-editing. The predicted editing window of both ABEs spanned positions 4 to 7 in the spacer region; the highest observed editing levels were at position A5 with 67% (ABE7.10-SpRY), 53% (splitABE+Rapamycin) and 10% (splitABE+DMSO), translating to a 5.1-fold increase in editing efficiency in the presence of rapamycin. Despite the low level of editing in the absence of rapamycin indicating spontaneous dimerisation, the 5.1-fold increase in the presence of rapamycin confirms these two fusion proteins can be used to regulate editing in a drug-dependent manner offering the possibility for temporal control of editing.

To assess if splitABE is capable of correcting W1282X without bystander-edits we are using two different cell models, the BCi-NS1.1 immortalized cell line that we can differentiate into all known lung cell types under air-liquid interface culture conditions, for which the W1282X cell line was previously developed in our lab, and the 16HBE14o-W1282X immortalized bronchial epithelial cell line.

Further characterisation of ABEs' editing efficiency and profile is currently being performed through an in-depth analysis of the editing events, accompanied by a fluorescence protein reporter cell model that we developed. This will lead to an understanding of the hypothetical reduced bystander-effect of splitABE as well as the dimerization capacity in the absence/presence of rapamycin for the correction of W1282X. Ultimately, the unique temporal control of editing with splitABE can lead to a safer gene editing approach.

[1]PMID:37649273

P74

Journey to the Centre of the Airway: tackling cystic fibrosis with cell-penetrating VP22-ABEs

J. Alves[1,2,3], C. M. Farinha[2], P. T. Harrison[3]

[1]Biosciences Institute, University College of Cork, Department of Physiology, Cork, Ireland, [2]BioISI-Biosystems and Integrative Sciences Institute, Faculty of Sciences, University of Lisbon, Lisbon, Portugal, [3]Division of Pulmonary Medicine, Cincinnati Children's Hospital, Cincinnati, OH, United States

Gene editing targeting the progenitor lung basal cells (BC) may have the potential to repopulate the lung pseudostratified epithelium (LPE) with cells expressing a functional CFTR protein for any CF-causing mutation. Whilst luminal epithelial cells (LEC) can be readily transfected with DNA or RNA molecules encoding gene-editing ribonucleoprotein (RNP) complexes, the complex architecture of the LPE causes a potential impediment to the direct targeting of BC. Considering this, we wanted to determine if DNA or RNA molecules delivered to the LEC could transfer their gene-editing encoded RNPs to the nuclei of BC cells below.

Inspired by a previous study using the HSV-1 VP22 cell-penetrating peptide[1], we developed VP22-GFP and VP22-adenine base editor (VP22-ABEs) fusion proteins. We hypothesise that VP22-ABE-RNPs may retain VP22's intrinsic nuclear localization and intercellular trafficking capacity, thus being capable of traveling through the LPE and successfully delivering gene-editing RNPs to BC.

Expression vectors encoding VP22-GFP, VP22-ABE8e(NG), and VP22-ABE9-SpRY, were designed using gBlocks containing a CMV promotor. Initial characterisation of pVP22-GFP in HEK293T cells showed high levels of GFP expression 48h post-transfection and a distinct nuclear localisation pattern relative to the pGFP control, confirming the nuclear localisation capacity of VP22-GFP fusion proteins.

The base editing profile and editing window of VP22-ABEs and parental ABEs was characterised using 6 different gRNAs targeted to regions of the human genome present in HEK293T cells. The editing window of ABE8e(NG) and VP22-ABE8e(NG) covers positions 2 to 12 in the spacer region; the highest editing levels achieved at adenine in position 5 (A5) with 76.0 and 64.4%, respectively, and moderate levels in positions A3 to A7. Differently, the editing window of SpRY-ABE9 and VP22-SpRY-ABE9 only includes positions 5 to 6 in the spacer region; with slightly lower editing levels achieved at A5 with 53.8 and 45.7%, respectively, yet with a positive pay-off of minimal levels of editing at A6.

To explore the intercellular trafficking capacity of VP22-ABEs between co-culture models, we developed a mCherry-Q47X fluorescence reporter cell model. We will electroporate HEK293T cells with VP22-ABEs and a gRNA-specific for the PTC in the mCherry-Q47X reporter. Co-culture of electroporated HEK293T cells and untransfected mCherry-Q47X will allows evaluation of our hypothesis that the VP22-ABE-gRNA-RNP complex can shuttle from the producer HEK293T cells into the neighbouring mCherry-Q47X reporter cell line and edit the PTC, thus demonstrating that VP22's intrinsic intercellular trafficking activity can shuttle ABE-gRNA complexes. Moreover, we will assess the VP22-ABEs intercellular trafficking and editing capacity in differentiated air-liquid interface (ALI) cultures, such as the BCi-NS1.1 with the W1282X variant which we have shown can be fully-differentiated into different cell types of the LPE. We will determine if VP22-ABE-RNP complexes produced in LEC can shuttle to the nuclei of BC in this model and functionally correct the W1282X variant using fluorescence-activated cell sorting to select for $NGFR^{+ve}$ BC; a substantial difference between the levels of VP22-ABEs *versus* parental-ABEs between the entire pool of cells and BC cells only, would be indicative of successful shuttling and editing capacity of VP22-ABE-RNP complexes into BC.

[1]PMID:10341888

P75

Development of GE-vesicles as CRISPR-Cas delivery system in genome editing applications for cystic fibrosis

G. Maule, M. Stancampiano, E. Gurrieri, F. Zerbini , A. Cereseto

University of Trento, CIBIO, Povo (Trento), Italy

Genome editing technologies hold great promise as potential tools for correcting CFTR mutations in cystic fibrosis (CF). Several studies have demonstrated the efficient recovery of mutated CFTR in experimental models of CF. However, current delivery methods remain inefficient, posing a major barrier to their clinical translation, particularly for targeting the lungs—the primary site of morbidity and mortality in CF patients. Engineered vesicles are promising delivery systems for genome editing tools offering a transient expression of the editing system to prevent side effects generated by their long-term expression and tuneable tissue tropism.

In this study, we developed genome editing vesicles, named GE-vesicles, a new and effective system to deliver genome editing technologies as ribonucleoprotein complexes to target airway epithelial cells.

We are currently focused on the delivery of base editors, specifically ABE8e-SpCas9, due to their proven efficiency in precisely and effectively repairing CFTR mutations (Amistadi et al., 2023; Umbach et al., personal communication). The GE-vesicles were produced using membrane anchoring motifs to capture maximal amounts of ABE8e-SpCas9 and sgRNA. Particles were characterized for size, particle number and editor content showing overall homogenous size in the range of 100-150 nm and efficient encapsulation of the ABE8e-SpCas9. To test the efficacy of the GE-vesicles we used different sgRNAs targeting CFTR exons in HEK293T and CFBE41o- cell lines. We obtained up to 60% of base conversion proving GE-vesicles as an efficient delivery system. We are currently testing GE-vesicles to correct some of the most frequent CF-causing nonsense mutations (R553X, R1162X and W1282X).

The envelope used to deliver GE-vesicles in target cells is the vesicular stomatitis virus-G protein (VSV-G) widely used with retroviral vectors and virus-like particles delivery systems. Even though VSV-G is a pantropic envelope, it has been reported to inefficiently target the apical side of differentiated epithelial cells. We are thus currently evaluating envelopes derived from different viruses aiming at efficient delivery in differentiated epithelial cells.

Overall, the GE-vesicles lay the foundation for editing tools delivery, which will be further adapted for targeted delivery to lung epithelial cells.

P76

Predicting benchmark efficiency for CF gene therapies using 3D analyses of individual mosaic organoids

J. Bulkens[1], A. Griffioen[1,2], N. Ithaksiou[1,2], M. Smith[1,2,3], S. van Beuningen[1,2,3], J. Beekman[1,2,3], E. Kuijk[1,2]

[1]UMC Utrecht, Pediatric Pulmonology, Utrecht, Netherlands, [2]Regenerative Medicine Center, Utrecht, Netherlands, [3]Centre for Living Technologies, Utrecht, Netherlands

Gene therapy promises to tackle the root cause of disease and a cure for people with cystic fibrosis. Several research groups have shown rescue of function for multiple prevalent genetic CFTR-variants in vitro and in vivo. However, transferring these results to the clinic remains challenging as many clinical trials for CF gene therapy have failed. This lack of success is probably due to low efficiencies in restoring CFTR function in disease-relevant cells. An important question is how many cells with restored CFTR function are required to compensate for the lack of CFTR function in the remaining cells.

In this study we aim to uncover the benchmark that determines desired gene therapy efficiency. We developed a mosaic organoid model of the human airway epithelium, which is composed of CFTR proficient and CFTR deficient patient-derived cells. Primary and immortalized basal cell cultures were transduced with lentivirus followed by antibiotic selection or FACS-sorting to create stable lines with green and red fluorescent nuclei. This enabled the mixing of red and green cells with different CFTR genotypes that differentiated in air-liquid interface (ALI) cultures and gave rise to mosaic organoids. These mosaic organoids show a broad range of genetic composition as determined by AI-based segmentation of the red and green fluorescent nuclei. This dataset allows us to determine the relationship between organoid size and genetic composition before and after forskolin-induced swelling (FIS).

We anticipate that enlarging our data set will help us to predict the benchmark value of the proportion of healthy cells that is required to restore overall CFTR functionality of individual organoids towards healthy levels.

We will exploit our large biobank containing CFTR deficient and proficient airway and intestinal cell lines to create mosaic organoids with a broad range of healthy/diseased cell ratios of different genotypes. Furthermore, we will combine our FIS assays with drug screening to assess whether synergy between gene therapy and conventional therapeutics can be observed. Altogether we expect that our study on mosaic organoids with mixed CFTR backgrounds will allow us to determine benchmark levels for gene therapies for CF.

This benchmark efficiency can strongly influence decision making regarding gene editing or supplementation molecules, the delivery vehicles of these molecules, and route of administration.

P77

A multicellular modelling framework for airway epithelial fluid and ion transport: implications for cystic fibrosis gene therapy and building towards a functional atlas

O. Hamed[1], G.W. Mos[2], V. Dua[1]

[1]University College London, Department of Chemical Engineering, London, United Kingdom, [2]University College London, Department of Neuroscience, Physiology and Pharmacology, London, United Kingdom

Epithelial ion transport regulates the depth of airway surface liquid (ASL), a thin fluid layer (0.1-10 μm) lining the airway epithelium, enabling vital clearance of mucus-trapped pathogens in the upper airways. ASL homeostasis is regulated by a complex collaboration of ion channels, transporters, and tight junction proteins. In cystic fibrosis (CF), where this system is disrupted, the ASL becomes dehydrated creating sticky mucus that is difficult to clear, resulting in chronic lung infections.

Existing mathematical models of ASL regulation typically treat the system as a single, idealised cell type containing all the important elements of fluid/ion transport. However, airway epithelia comprise patchworks of distinct cell types, each with specialised functions. To explore the implications of this for ASL homeostasis, we developed a multicellular quantitative framework to understand the bioelectric properties of healthy and CF epithelia and, in the latter case, the implications of cell diversity for gene therapy.

We previously presented a two-cell-type model highlighting a key role for the $Na^+/K^+/2Cl^-$ cotransporter (NKCC) and basolateral Cl^- channels (ClC_{ba}). A cell expressing ClC_{ba} in the absence of NKCC resulted in a cell absorbing Cl^- from the ASL, while a neighbouring cell with NKCC present, and ClC_{ba} absent, secretes Cl^-. Both cell types were modelled expressing apical CFTR, thus supporting a cell type-dependent role for CFTR in both Cl^- absorption and secretion.

Taking this model further, we simulated CF epithelia and subsequent gene delivery in multiple scenarios. The overall ambition of our modelling framework is to create a functional atlas for human airway epithelial cell ion transport, capable of predicting gene delivery outcomes when strong or weak promoters are used, or CFTR polarization is imperfect. We conclude that such models can help to elucidate the roles of different cell types in ASL regulation and inform the design and development of successful gene therapies.

P78

A novel LNP formulation for the targeted delivery of gene editing tools to lung epithelial cells

S. Saxena[1], S. Hak[2], A. Colliva[1], S. Vodret[1], J. Parot[3], A. Molska[3], G. Maule[4], E. Guerrieri[4], M. Stancampiano[4], S.E. Borgos[2], A. Cereseto[4], S. Zacchigna[1]

[1]International Centre for Genetic Engineering and Biotechnology, Cardiovascular Biology Laboratory, Trieste, Italy, [2]SINTEF, Department of Biotechnology and Nanomedicine, Trondheim, Norway, [3]SINTEF, Trondheim, Norway, [4]University of Trento, Centre for Integrative Biology (CIBIO), Trento, Italy

Introduction: Obstructive pulmonary function leading to chronic inflammation and lung failure is the most common cause of mortality in cystic fibrosis (CF). Recent successes in genome editing have opened new avenues for selectively targeting CFTR mutations in lung epithelium. Efficient and specific delivery of the editing machinery to lung epithelial cells remains a major challenge.
Methods: Our consortium aims to develop non-viral gene transfer tools to deliver editing tools to the lung. Here, we report about a novel lipid nanoparticle (LNP) formulation (GD19) that target different cell types in the lung, depending on the route of administration. To permanently label transfected cells we encapsulated Cre-mRNA into GD19 LNPs and delivered them to Rosa26-mTmG mice, which express membrane-targeted tdTomato before Cre excision and membrane-targeted GFP following Cre excision. LNPs were delivered via either intratracheal (IT) administration in adult mice or intravenous (IV) injection in neonates. After 72 hours, lungs, liver and heart were collected and processed for immunofluorescent staining.
Results: High levels of Cre-mediated recombination were observed, resulting in mGFP expression throughout the lung parenchyma upon both administration routes. IT administration resulted in a high percentage of CFTR-expressing airway epithelial cells transfected with LNPs. Nearly 80% of GFP-positive cells co-expressed uteroglobin, (SCGB1A1, a marker for secretory cells), 48% co-expressed SOX2 (a marker for epithelial cells in both airways and submucosal glands), and 75% co-expressed NKX2.1 (a marker for bronchial epithelial and alveolar type 1 cells). Approximately 20% of mGFP-positive cells also expressed Prosurfactant C (a marker for alveolar type 2 cells), indicating that IT administration transfected not only airway epithelial cells but also interstitial alveolar cells.
In contrast, IV administration of the same GD19 LNP formulation resulted in the transfection of predominantly endothelial and other interstitial cells, with minimal targeting of airway epithelial cells. Additionally, by IV administration we observed high transfection efficiency in both liver (almost 100% of the cells were positive for mGFP) and heart (primarily endothelial cells). This point to IT delivery as the best delivery route for targeting epithelial cells critical for CF therapy.
Conclusions: In conclusion, our GD19 LNP formulation efficiently delivered mRNAs to lung epithelial cells that physiologically express high levels of CFTR. Ongoing work is assessing their efficacy in delivering base editor tools as a novel therapeutic opportunity for CF.