2026

European Cystic Fibrosis Society

21st ECFS Basic Science Conference

Conference Programme & Abstract Book

Malta

Chairpersons

Batsheva Kerem, Isabelle Sermet and Jennifer Bomberger

11 March - 14 March 2026

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WELCOME FROM THE ECFS PRESIDENT

I am happy to welcome you to the 21st edition of the ECFS Basic Science Conference, set against the stunning backdrop of St. Paul's Bay, Malta.

This annual gathering continues to be a cornerstone for researchers dedicated to advancing our understanding of cystic fibrosis. This year, we are enhancing the programme with new elements. Alongside our hallmark symposia and abstract-driven presentations, we are introducing several sessions designed to broaden engagement and spark fresh dialogue.

We remain deeply grateful to our senior colleagues whose ongoing contributions and mentorship continue to shape the field. Your presence and insights are integral to the collaborative spirit of this conference, and we look forward to the rich discussions your experience brings.

I would especially like to thank Batsheva Kerem for her leadership as conference chair, and to Isabelle Sermet and Jennifer Bomberger for their dedicated support as co-chairs.

As always, the conference remains a vibrant space for exchanging research, exploring new ideas, and fostering international collaboration in an informal, interactive setting.

A very warm welcome to what promises to be an inspiring and forward-thinking conference.

Jane Davies
President
European Cystic Fibrosis Society

WELCOME FROM THE CONFERENCE CHAIRPERSONS

We are delighted to welcome you to the 21st Basic Science of CF Conference, here in St Paul's Bay, Malta. This year's meeting brings us together in a setting shaped by centuries of exchange and resilience - qualities that resonate with the collaborative spirit of our field. Malta's unique blend of history, culture, and coastal landscape provides a fitting backdrop for a conference dedicated to discovery and innovation.

At the heart of this meeting is the science. Over the coming days, you will hear cutting-edge, often still unpublished work spanning molecular mechanisms, cellular biology, emerging technologies, and novel therapeutic approaches in cystic fibrosis. As always, the conference is designed to encourage open discussion, critical thinking, and direct interaction across all career stages.

A defining strength of this event is the high participation of early-career researchers who contribute enormously through their energy, ideas, and willingness to challenge established assumptions. We encourage everyone to engage fully, share data, debate constructively, and build connections that will shape future collaborations.

This year's programme features eight symposia with invited and selected speakers, two keynote lectures, our popular flash-poster sessions and the new Young Investigators Programme. Together, these sessions provide a unique opportunity to explore current questions and emerging directions in basic CF research with colleagues from across Europe and beyond.

We warmly welcome scientists from diverse fields whose work intersects with cystic fibrosis. Your perspectives enrich the discussions and advance our shared goal of deepening fundamental understanding to ultimately improve patient care.

We wish you an inspiring and stimulating conference in Malta.

Batsheva Kerem
The Hebrew University
Jerusalem
Israel

Isabelle Sermet
Paris Cite University
Paris
France

Jennifer Bomberger
Geisel School of Medicine
at Dartmouth
Hanover
USA

21st ECFS Basic Science Conference

11 – 14 March 2026, St Paul's Bay, Malta

Programme

Chairpersons: Batsheva Kerem (Jerusalem, Israel), Isabelle Sermet (Paris, France) & Jennifer Bomberger (Hanover, US)

Wednesday, 11 March 2026 (Day 1)	
14:30-17:30	**Young Investigators Workshop**
	Career Development Session & Networking Reception
17:45-18:15	**Official Opening of the Conference by the Conference Chairpersons**
18:15-19:15	**Opening Keynote Lecture:** Mucus, inflammation and infection in the modulator era – Marcus Mall (DE)
19:15-20:00	**Welcome Reception**
20:00-21:30	*Dinner*

Thursday, 12 March 2026 (Day 2)	
07:30-08:45	*Breakfast*
08:45-10:30	**Symposium 1 – Nonsense Mutations & Translational Correction**
	Chairs: Batsheva Kerem (IL) / Kathryn Oliver (US)
08:45-09:10	Let's put a stop to this nonsense - gene editing strategies for PTC variants – Patrick Harrison (US)
09:10-09:35	Suppression of CFTR nonsense mutations by distinct classes of small molecule readthrough agents – David Bedwell (US)
09:35-10:00	Engineered suppressor transfer RNAs for efficient suppression of pathogenic nonsense mutations – Jeffrey Tharp (US)
10:00-10:10	Abs. 68 - A unique combination of nucleotide modifications enhances suppressor tRNA efficacy in restoring CFTR function – Stine Behrmann (DE)
10:10-10:20	Abs. 91 - Rescue of the CFTR chloride channel with premature termination codons is markedly improved under inflammatory conditions – Anna Borrelli (IT)
10:20-10:30	Abs. 11 - Correction of CFTR nonsense mutations by SRI-41315 alone or combined with Alyftrek – Frédéric Becq (FR)
10:30-11:00	*Coffee break* & **Poster viewing**
11:00-12:45	**Symposium 2 – Gene Therapy & Genome Editing**
	Chairs: Jeffrey Beekman (NL) / Marianne Carlon (BE)
11:00-11:25	Challenges and progress in the delivery of gene editors to airway epithelial cells – Paul McCray (US)
11:25-11:50	Highly efficient replacement of the *CFTR* cDNA to treat all people with cystic fibrosis – Sriram Vaidyanathan (US)

11:50-12:15	Virus-like particles for ‘hit and go’ gene editing to treat the cystic fibrosis gene defect – Marianne Carlon (BE)
12:15-12:25	Abs. 70 - Silencing the silencers: antisense oligonucleotides targeting intronic splicing silencer motifs in CFTR – Mark Leahy (PT)
12:25-12:35	Abs. 83 - Functionalized nonsense suppressor tRNA picovectors represent a novel therapeutic cargo for the treatment of PTC-associated CF – Joseph Porter (US)
12:35-12:45	Abs. 87 - Engineered virus-like particles successfully deliver adenine-base editing to restore CFTR function by correction of R553X – Martina Scallan (IE)
12:45-14:15	*Lunch*
14:15-16:00	**Symposium 3 – Inter-organ Crosstalk & Physiology**
	Chairs: Alison Kohan (US) / Isabelle Sermet (FR)
14:15-14:40	Lipid metabolism dysfunction in CF via the gut-liver axis – Alison Kohan (US)
14:40-15:05	Does the gut-lung axis play a role in cystic fibrosis disease? – Lucas Hoffman (US)
15:05-15:30	Extracellular vesicles in chronic muco-obstructive pulmonary diseases: role in pathogenesis and as biomarkers – Massimo Conese (IT)
15:30-15:40	Abs. 12 - Cystic fibrosis airway epithelium secretome profiling: new insights into CFTR-related dysregulations – Emilie Luczka Majérus (FR)
15:40-15:50	Abs. 61 - Cystic fibrosis associated colorectal cancer: linking CFTR-deficiency to the increased risk of developing CRC – Maud van der Wijst (NL)
15:50-16:00	Abs. 36 - Neutrophil transmigration across cystic fibrosis bronchial epithelia is modulated by hyperglycemia – Guiying Cui (US)
16:00-16:30	*Coffee break* & **Poster viewing**
16:30-17:30	**Flash Poster Session (even numbers)** **Chair: Lucas Hoffman (US)**
17:30-18:00	**ECFS Working Groups in Focus** **Chair: Jane Davies (UK)** Airway Epithelial Cell Models for Theranostics – Nicoletta Pedemonte (IT) Intestinal Organoids – Sacha Spelier (NL)
18:00-19:30	**Evening Poster Session:** Posters with even numbers
20:00-21:30	*Dinner*

Friday, 13 March 2026 (Day 3)	
07:30-08:45	*Breakfast*
08:45-10:30	**Symposium 4 – Metabolic Dysfunction in CF**
	Chairs: Jennifer Bomberger (US) / Sebastián Riquelme (US)
08:45-09:10	CFRD modifies the sputum microbiome: implications for HEMT – Deborah Baines (UK)

09:10-09:35	Mitochondrial alterations that shape CF lung infection – Sebastián Riquelme (US)
09:35-10:00	Early life pancreatic remodeling in CF ferrets – John Engelhardt (US)
10:00-10:10	Abs. 38 - Metabolic regulation of pulmonary infection trajectory in cystic fibrosis – Jenny Tuyet-Nhung Truong (DE)
10:10-10:20	Abs. 18 - A secretory cell is never late: CFTR emerges early in epithelial regeneration – Aditi Shekhar (NL)
10:20-10:30	Abs. 7 - Targeting a PI3Kγ–AKAP13–PKD1 signaling axis enhances F508del-CFTR trafficking and potentiates ETI therapy – Marco Mergiotti (IT)
10:30-11:00	*Coffee break* & **Poster viewing**
11:00-12:45	**Symposium 5 – Vascular and Cardiovascular complications** a patient organisation-initiated symposium
	Chairs: Margarida Amaral / Robert Gray
11:00-11:10	CF community survey results – Paula Sommer (UK)
11:10-11:40	Cardiovascular risk in cystic fibrosis in the modulator era: emerging evidence and unanswered questions – Damian Downey (UK)
11:40-12:10	Vascular changes in the end-stage CF lung – what is the link with CFTR? – Mieke Boon (BE)
12:10-12:40	Imaging the brain in people with cystic fibrosis – Hannah Chandler (UK)
12:40-12:45	Wrap Up – Paula Sommer (UK)
12:45-14:15	*Lunch*
14:15-18:30	**Free Afternoon**
18:30-19:30	**Flash Poster Session (odd numbers)** **Chair: Deborah Baines (UK)**
19:30-19:45	The Italian CF Research Foundation – Ermanno Rizzi (IT)
20:00 -21:30	*Dinner*
21:30-23:00	**Evening Poster Session:** Posters with odd numbers
Saturday, 14 March 2026 (Day 4)	
07:30-08:45	*Breakfast*
08:45-10:30	**Symposium 6 – Organ-on-a-Chip & New Models**
	Chairs: Jennifer Bomberger (US) / Roberto Plebani (IT)
08:45-09:10	Organ-on-a-Chip technology for modeling human lung pathophysiology – Roberto Plebani (IT)
09:10-09:35	The bronchioid model: a tool to recapitulate features of distal airways and disease-relevant phenotypes – Isabelle Dupin (FR)
09:35-10:00	Complex airway models to study infection in CF – Helle Krogh Johansen (DK)
10:00-10:10	Abs. 71 - Targeting CFTR, K^+ channels, and bacterial virulence to restore airway epithelial integrity in preclinical cystic fibrosis models – Emmanuelle Brochiero (CA)

10:10-10:20	Abs. 56 - Advanced endometrial organoid models to unravel endometrium-linked fertility problems in cystic fibrosis – Beau Gommers (BE)
10:20-10:30	Abs. 57 - Organotypic in vitro model of the human lung using hiPSCs to study epithelial-macrophage interactions and bacterial infections of the upper respiratory tract in cystic fibrosis – Ruth Olmer (DE)
10:30-11:00	*Coffee break* & **Poster viewing**
11:00-12:45	**Symposium 7 – Systems Biology & Multi-omics and CFTR structure**
	Chairs: Margarida Amaral (PT) / Véronique Stoven (FR)
11:00-11:25	Beyond the ion channel: systems-level insights into CFTR and epithelial transformation – Margarida Amaral (PT)
11:25-11:50	Molecular mechanisms of CFTR channel regulation by protein kinase A – Lászlo Csanády (HU)
11:50-12:15	Omics data and systems biology approaches in the context of CF – Véronique Stoven (FR)
12:15-12:25	Abs. 3 - Exploring the cellular pathways to promote rescue of mutant CFTR protein in cystic fibrosis – Cláudia Alves (PT)
12:25-12:35	Abs. 9 - ATP-dependent modulation of the NBD1 conformational equilibrium from the CFTR channel – Arina Svoeglazova (BE)
12:35-12:45	Abs. 100 - Systemic effects of cystic fibrosis transmembrane conductance regulator (CFTR) modulators on the plasma and serum proteome – Kerstin Fentker (DE)
12:45-14:15	*Lunch*
14:15-16:00	**Symposium 8 – Infection and Immunology in the CFTR modulator era**
	Chairs: Robert Gray (UK) / Katrine Whiteson (US)
14:15-14:40	Adaptation of *Staphylococcus aureus* during chronic infection of the lung – Anne Jamet (FR)
14:40-15:05	Is inflammation still a target in cystic fibrosis? – Robert Gray (UK)
15:05-15:30	Reimagining infection management in CF with phage therapy: from biology to clinical application – Katrine Whiteson (US)
15:30-15:40	Abs. 96 - The PD-1 / SHP2 immune checkpoint controls bacterial killing by human CF airway neutrophils – Rabindra Tirouvanziam (US)
15:40-15:50	Abs. 41 - Long-standing adaptation and regulatory plasticity in *Pseudomonas aeruginosa* during CFTR restoration – Cristina Cigana (IT)
15:50-16:00	Abs. 45 - ETI modulates airway inflammation and reduces *Pseudomonas aeruginosa* burden in pediatric cystic fibrosis – Feriel Kanoun (FR)
16:00-16:30	*Coffee break* & **Poster viewing**
16:30-17:45	**Conference Closing**
	Closing Keynote lecture Ethics in research: feedback from the bench – Bruno Clement (FR)
20:00	*Dinner & Social Event*

POSTERS TITLES & AUTHORS

P 1 ATP-independent activity of elexacaftor-tezacaftor-ivacaftor-rescued F508del-CFTR
J.N. Charlick, M. Rodrat, D.N. Sheppard

P 2 Impact of elexacaftor-tezacaftor-ivacaftor on pig F508del-CFTR, a Cl- channel with greater residual activity than human F508del-CFTR
D.R.S. Ng, M. Rodrat, J.N. Charlick, D.N. Sheppard

P 3 Exploring the cellular pathways to promote rescue of mutant CFTR protein in cystic fibrosis
C. Alves, J. Oliveira, J. F. Ferreira, A. di Fonzo, A. Armirotti, N. Pedemonte, V. Tomati, C.M. Farinha

P 4 Revertant mutations in nucleotide binding domain 1 nearly restore the protein processing and channel gating of ΔF508-CFTR
M. Wu, M. Cao, Y. Zhi, D. Fasseho, Y. Jin, J.-H. Chen

P 5 Protein and small molecule mediators of the rescue of p.Phe508del-CFTR through GRK5 inhibition
M.F Caleiro, D. Neugebauer, S. Abdul, I. Michou, R. Pacheco, M.D Amaral1, H.M Botelho

P 6 Probing the structure of human-mouse CFTR chimeras using molecular modelling with AlphaFold3
Z. Liu, Z. Cai, D. Lea-Smith, B. J. Stevenson, A. Doherty, H. Davidson, D. J. Porteous, I. Callebaut, R. A. Corey, D. N. Sheppard, A.C. Boyd

P 7 Targeting a PI3Kγ–AKAP13–PKD1 signaling axis enhances F508del-CFTR trafficking and potentiates ETI therapy
M. Mergiotti, A. Murabito, V. Capurro, A. Loffreda, M. Li, P. Peretto, A. Raimondi, C. Tacchetti, D. Diviani, N. Pedemonte, E. Hirsch, A. Ghigo

P 9 ATP-dependent modulation of the NBD1 conformational equilibrium from the CFTR channel
A.Svoeglazova, M. Overtus, T. Kache, C. Martens, J. Hendrix, C. Govaerts

P 10 Investigating promiscuity of elexacaftor and GoSlo: can we better understand the mechanism of action ?
J.N. Lunavath, O. Hamed, D.C. Benton, G.W. Moss, P. Vergani

P 11 Correction of CFTR nonsense mutations by SRI-41315 alone or combined with Alyftrek
L. Damy, S. Mirval, C. Barrault, F. Becq

P 12 Cystic fibrosis airway epithelium secretome profiling: new insights into CFTR-related dysregulations
E. Luczka Majérus, A. Brisebarre, C. Dos Santos - Dietz, J. Cellier, C. Kileztky, A. Bonnomet, F. Delalande, E. Sage, K. Bessaci-Kabouya, M. Polette, L. Sabatier, C. Coraux

P 13 Intestinal CFTR-mediated chloride currents do not parallel bicarbonate secretion rates in CF
M. Barillaro, J. Pan, J. Avolio, T. Gonska

P 14 Airway HCO3 - and H+ secretion: role of pulmonary ionocytes
J.W. Hanrahan, D. Kim, N. Scales, D. Cruiziat, Y. Luo, J. Alizadehnohi

P 15 Impact of low molecular weight hyaluronic acid on airway epithelial remodelling in cystic fibrosis
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P 16 Investigating TMEM16A and TRPV4 as calcium-signaling-related proteins involved in airway hydration and defense mechanisms in cystic fibrosis
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P 17 Investigation of CFTR function and epithelial barrier properties at single cell resolution using multi-electrode array technology
M.M. Ensinck, A. Venz, A.S. Ramalho, G. Amatngalim, S. Ebrahimi Takalloo, F. Vermeulen, D. Braeken, M.S. Carlon

P 18 A secretory cell is never late: CFTR emerges early in epithelial regeneration
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P 19 Identification of novel SLC26A9 inhibitors for the study of non-CFTR mediated anion secretion
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P 20 Pediatric cystic fibrosis with CFRD and celiac disease: contribution of TCF7L2 rs7903146 (TT) and rs4077468 (GG) variants in disease complexity
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P 21 Adipogenesis, a key factor affecting bone health in patients with cystic fibrosis
L. Hamon, C. Dumortier, J. Sergheraert, S. Gangloff, D. Al Alam, J. Braux, M.-L. Jourdain, F. Velard

P 22 The control of airway surface liquid pH in cystic fibrosis (CF) and non-CF airways
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P 23 Elexacaftor/Tezacaftor/Ivacaftor (ETI) restores bicarbonate transport of F508Del-CFTR
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P 27 CFTR correction, as a new therapeutic option in the treatment of chronic pancreatitis
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P 28 IL-17A and IL-17F differentially modify ion transport and apical surface properties in human bronchial epithelia
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P 29 Epigenome-wide association study and functional analyses reveal regulatory loci linked to lung function and diabetes in cystic fibrosis
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P 30 Hypoxia and inflammation drive distinct signalling pathways in human airway epithelia
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P 37 Microbial crosstalk: influence of Pseudomonas aeruginosa on Mycobacterium abscessus virulence and antibiotic tolerance
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P 38 Metabolic regulation of pulmonary infection trajectory in cystic fibrosis
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P 41 Long-standing adaptation and regulatory plasticity in Pseudomonas aeruginosa during CFTR restoration
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P 44 Pseudomonas aeruginosa induced GM-CSF production by airway epithelial cells could help defend against Mycobacterium abscessus
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P 45 ETI modulates airway inflammation and reduces Pseudomonas aeruginosa burden in pediatric cystic fibrosis
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P 46 De novo pyrimidine reprogramming shapes cystic fibrosis lung disease
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P 47 Leveraging metabolic signatures induced by CFTR modulator therapy to eradicate lung infection
S. Roy, G. Lohia, Y.-T Chen, M. Zaman, S. Riquelme

P 48 Exhaled breath condensate analyses identify specific human protein signatures for detecting individual airway pathogens in people with cystic fibrosis
M. Salathe, M.D. Kim, C.D. Bengtson, M.J. Rekowski, N. Baumlin, S.H. Bossmann, M.E. Sardiu, M.P. Washburn

P 49 CFTR protein expression in epithelial tissues of a 2XHA-CFTR mouse model
C. Flores, F. Castillo, S. Villanueva, S. Hernández-Rivas, I. Cornejo

P 50 Application of a semi-automated image analysis algorithm and respiratory organoids for cystic fibrosis drug screening
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P 53 Uncovering how ETI therapy reshapes chronic Pseudomonas infection in primary cystic fibrosis bronchial epithelial cultures
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P 54 Preclinical model of precision cut lung slice for the evaluation of therapeutic treatments in CF
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P 55 An iPSC-derived airway organoid approach to evaluate potential CFTR modulator benefits in an individual with CF carrying rare compound heterozygous mutations using the FIS assay
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P 56 Advanced endometrial organoid models to unravel endometrium-linked fertility problems in cystic fibrosis
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P 57 Organotypic in vitro model of the human lung using hiPSCs to study epithelial-macrophage interactions and bacterial infections of the upper respiratory tract in cystic fibrosis
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P 58 Implementation of in vitro and ex vivo approaches to study the impact of CFTR dysfunction on osteocytes in CFBD
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P 59 Modeling Pseudomonas aeruginosa biofilm and phage therapy in a cystic fibrosis ex vivo pig lung model
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P 60 Addressing cystic fibrosis inequality in low and middle income countries: a role for patient-derived intestinal organoids
S. Kroes, J. Taylor-Cousar, M. Zampoli, B. Karadag, K. van der Ent, K. de Winter - de Groot, J. Beekman, S. Spelier

P 61 Cystic fibrosis associated colorectal cancer: linking CFTR-deficiency to the increased risk of developing CRC
M.I.M. van der Wijst, V.J. Niessen, D. Muilwijk, R.W. Hofland, J.M. Beekman, S. Spelier

P 62 Cystic fibrosis sputum enhances neutrophil motility in 3D collagen matrices
M. Garcia Gomez, I. Poe, M. Bernard, A. Deslys, E. Bardan, E. Tejerina, F. Kanoun, A. Hinzpeter, I. Sermet, P. Vargas

P 63 Illuminating CF drug discovery via fluorescent organoid-derived screening models
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P 64 From Cells to Breath: Identifying therapeutic biomarkers using in vitro volatilomics
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P 65 Using air–liquid interface airway cultures to study early *Pseudomonas aeruginosa* infection behaviours during CFTR modulator therapy
M. Pals, S. Lolle, S. Molin, H.K. Johansen

P 66 Modeling class I mutation with CRISPR/Cas tools: hiPSC-derived organoids in cystic fibrosis
B.Simonneau, S. Mienanzambi, O. Ruckebush, B. Vrablikova, M. Cailleret, S. Baghdoyan, V. Fournier, B. Louis, P. Fanen, B. Duriez

P 67 Of mice and men: Decoding the genomic background of βENaC-Tg mouse model through whole genome sequencing
C.Black, J. Alves, A. Husami, M. Duszynski, M. Imbrogno, R. Pasula, K. Hudock, P. Harrison

P 68 A unique combination of nucleotide modifications enhances suppressor tRNA efficacy in restoring CFTR function
S. Behrmann, D. Joshi, L. Höeg, E.J. Sorscher, Z. Ignatova, S. Albers

P 69 Safely skipping stops: a proteomics-based framework for evaluating readthrough therapies in cystic fibrosis
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AWARD WINNERS

ECFS Young Fellows Travel Award

Joanna Alves (IE)
Marjolein Ensinck (BE)
Mark Leahy (PT)
Zhujun Liu (UK)
Sweta Roy (US)
Tihomir Rubil (DE)
Aditi Shekhar (NL)
Léa Thoraval (FR)

Student Helpers

Jasmin Berger (DE)
Julie Cellier (FR)
Beau Gommers (BE)
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Young Researchers Registration Grant
Supported by the Italian Cystic Fibrosis Research Foundation

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American Young Investigator Travel Grant
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Ella Burns
Molly Duszynski
Gaurav Lohia

Canadian Trainee Registration Grant
Supported by CF Canada

Manilla Barillaro

11 March - 18:15 - 19:15

Opening Keynote Lecture

Mucus, inlammation and infection in the modulator era

Marcus Mall

Department of Pediatric Respiratory Medicine, Immunology and Intensive Care Medicine
Charité Universitätsmedizin Berlin
Berlin, Germany

No abstract submitted.

12 March - 08:45 - 10:30

Symposium 1
Nonsense Mutations & Translational Correctoin

S 1.1 Let's put a stop to this nonsense - gene editing strategies for PTC variants

Patrick Harrison

Cincinnati Children's Hospital, Cincinnati, Ohio, USA

There are published gene editing strategies for the five most common CF-causing PTC variants yet none have been tested clinically.

This talk will explore the different editing and delivery modalities used to date, and discuss the options and feasibility of developing editing strategies for all known CF-causing PTCs.

In addition, the talk will outline some of the steps to be considered in translation of this approach to a clinical trial, and the hurdles to be cleared on the way to this goal.

S 1.2 Suppression of CFTR nonsense mutations by distinct classes of small molecule readthrough agents

David M. Bedwell[1,3], Jianguo Chen[1,2], Ming Du[1,3], Ping Ye[3], Omar Moukha-Chafiq[3], Vinson, Paige[4], Sixue Zhang[5], Lianwu Fu[1,3], Kim M. Keeling[1,3], Feng Liang[6], Kevin Coote[6], Hermann Bihler[6], Martin Mense[6], Steven M. Rowe[1,2], and Corinne E. Augelli-Szafran[1,3].

[1]The Gregory Fleming James Cystic Fibrosis Research Center and the Departments of [2]Medicine, [3]Biochemistry and Molecular Genetics, University of Alabama at Birmingham (UAB), Birmingham, AL, [4]Scientific Platforms Division, Southern Research, Birmingham, AL. [5]System Pharmacology AI Research Center, UAB, Birmingham, AL. [6]Cystic Fibrosis Foundation Therapeutics (CFFT) Lab, Cystic Fibrosis Foundation (CFF), Lexington, MA

Background: Premature termination codons (PTCs) affect ~11% of individuals with CF, yet effective therapies are limited. Translation termination occurs when a stop codon enters the ribosomal A site. The translation termination complex (made up of the translation termination factors eRF1, eRF3, and GTP) binds the stop codon in the A site and facilitates hydrolysis of the ester bond that connects the nascent chain bound to the P site peptidyl-tRNA. This releases the polypeptide from the ribosome. A low level of PTC suppression (termed readthrough) naturally occurs at a low frequency ($\leq$ 0.1%) during protein synthesis. However, studies over the last decade have identified small molecules that enhance suppression of PTCs and allow the enhanced translation of full-length protein. Here, we will describe five classes of readthrough molecules that promote the suppression of PTCs. These classes include: 1) aminoglycosides (AGs); 2) AG enhancers; 3) eRF1 degraders; 4) eRF3 degraders; and 5) eRF3 degrader enhancers.

Methods: Representative molecules from each of these mechanistic classes are being tested in a range of formats, including luciferase-based reporter assays, Trans-Epithelial Chloride Conductance (TECC) assays in 16HBEs, and western blots in a range of cell types, including: 1) 16HBEs; 2) Primary HBEs; and 3) Intestinal organoids.

Results: Representative molecules from each readthrough compound class enhance PTC readthrough in various cell types and assays, in some cases resulting in 5-10% of WT CFTR activity. We continue to optimize these classes based upon: 1) pharmacological properties; 2) efficacy, and 3) potency. We are also exploring combinations of these compounds to determine whether a multidrug therapy might be able to attain the level of CFTR PTC readthrough needed to provide a therapeutic benefit.

Conclusions: While still a work in progress, our program to develop an effective therapy to mediate suppression of CFTR PTC mutations continues to move forward. It is hoped that we will soon have one or more therapies ready to begin testing in clinical trials.

S 1.3 Engineered suppressor transfer RNAs for efficient suppression of pathogenic nonsense mutations

Caitlin Specht, Alejandro Tapia, Cody White, and Jeffery M. Tharp*

Department of Biochemistry, Molecular Biology, and Pharmacology, Indiana University School of Medicine, Indianapolis, IN 46202, USA

Nonsense mutations are genetic changes that convert an amino-acid-encoding codon into a premature termination codon (PTC). These mutations cause gene translation to terminate too early, producing a truncated protein that is often nonfunctional and rapidly degraded. Nonsense mutations contribute to a wide range of genetic disease, motivating strong interest in strategies that restore full-length protein synthesis without permanently altering the genome. Recently, engineered suppressor transfer RNAs (sup-tRNAs) have emerged as a promising approach to address nonsense-related diseases. sup-tRNAs are designed to recognize stop codons and insert an amino acid at PTC-encoded positions, effectively restoring full length protein synthesis. However, despite their potential, many sup-tRNAs exhibit relatively poor PTC suppression efficiency. This limitation has constrained their broader adoption and slow progress toward therapeutic translation. In this presentation, I will describe two complementary efforts in my lab to improve the efficiency of sup-tRNAs through rational design and directed evolution. In addition, I will present preliminary data demonstrating sup-tRNA-mediated correction of disease-relevant nonsense mutations implicated in breast and ovarian cancer and cystic fibrosis, highlighting both current capabilities and remaining challenges on the path to therapeutic application.

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S 1.4 A unique combination of nucleotide modifications enhances suppressor tRNA efficacy in restoring CFTR function

S. Behrmann[1], D. Joshi[2], L. Höeg[1], E.J. Sorscher[2], Z. Ignatova[1], S. Albers[1]

[1]Universität Hamburg, Institute of Biochemistry and Molecular Biology, Hamburg, Germany, [2]Emory University School of Medicine, Department of Pediatrics, Atlanta, United States

Approximately 10% of patients with cystic fibrosis harbor a nonsense mutation in the CFTR gene that confers a premature termination codon (PTC). This type of molecular defect results in the abrupt arrest of translation and loss of protein function. To correct PTCs, we have developed a novel strategy that repurposes sense-codon-decoding tRNAs into suppressor tRNAs (sup-tRNAs). These sup-tRNAs read through PTCs, thereby restoring full-length CFTR protein and functional activity (1, 2). We pursue two administration strategies: single-dose administration using episomal vectors and redosing of ready-to-use sup-tRNAs delivered by lipid nanoparticles. To minimize the need for redosing, we aim for maximum efficacy and intracellular stability of the sup-tRNAs. We identify a unique nucleotide modification that enhances the suppression activity of various sup-tRNAs up to 5-fold. We combine this modification with a secondary modification in the D-arm of the sup-tRNA, and show these changes can efficiently silence immunogenicity and blunt the innate immune response following sup-tRNA administration. The modified sup-tRNAs exhibit high stability, improving the therapeutic half-life. Modified sup-tRNAs restore CFTR activity of several disease-causing PTCs to levels above a threshold predicted to be therapeutic. In the single-dose approach, we modify the promoter-terminator tandem to achieve sup-tRNA levels comparable to the tissue-specific levels, causing no perturbation of the natural tRNAs. Notably, in difficult-to-suppress PTCs, such as G542X, we propose an alternative strategy, achieving substantial activity of CFTR in the FRT model – a cell system viewed as relevant to drug registration by the FDA.

(1) Albers, S. et al. Engineered tRNAs suppress nonsense mutations in cells and in vivo. Nature 618, 842-848.

(2) Bharti, N. et al. Translation velocity determines the efficacy of engineered suppressor tRNAs on pathogenic nonsense mutations. Nat Commun 15, 2957.

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S 1.5 Rescue of the CFTR chloride channel with premature termination codons is markedly improved under inflammatory conditions

A. Borrelli[1], A. Venturini[1], M. De Santis[1], D. Guidone[1], R. De Cegli[1], E. Montemitro[2], F. Alghisi[2], F. Ciciriello[2], L.J. Galletta[1,3]

[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, [3]University of Naples Federico II, Department of Translational Medicine, Naples, Italy

In cystic fibrosis (CF), nonsense mutations produce premature termination codons (PTCs) that hamper the correct CFTR protein translation. The resulting truncated forms of the CFTR chloride channel are insensitive to presently available CFTR modulators (correctors, potentiators). Read-through (RT) of the ribosome stalled at the PTC site by small molecules can allow continuation of the protein synthesis. ELX-02, a promising RT agent, completed phase II clinical trial for people with cystic fibrosis (pwCF). Besides RT agents, small molecules acting as eRF3a degraders, such as CC-90009, have been recently described as effective on PTCs. We evaluated the effect of CC-90009 in combination with ELX-02 on CFTR carrying PTCs. Previous works reported that TNFα and IL-17A treatment can enhance rescue of delF508-CFTR variant by CFTR modulators in human bronchial epithelial cells (HBECs). We investigated whether inflammatory stimuli could also improve pharmacological correction of PTCs.

We generated in vitro fully differentiated HBECs from a patient carrying the G542X mutation and we cultured them on porous supports under air-liquid interface (ALI) condition. We treated the cells with or without IL-4 or TNFα/IL-17A for 72 h. In the last 24 h, cells also received drug cocktails containing ELX-02 (200μM) + VX-809 (1μM), with or without CC-90009 (0.1 μM). We evaluated the rescue of CFTR function by short-circuit current (Isc) experiments, and protein and mRNA levels by automated capillary immunodetection and qRT-PCR, respectively. We performed bulk RNA-seq to assess gene expression changes under treatments.

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. Reduced SMG6 and UPF1 protein expression suggest that cytokine may limit nonsense mediated decay (NMD) process. Gene ontology analysis of RNA-seq data confirmed downregulation of NMD pathway.

Inflammatory stimuli significantly improve RT-based correction of G542X-CFTR. Dissection of the underlying mechanism could lead to identification of novel druggable molecular targets to rescue impairment of protein synthesis. Furthermore, pharmacological rescue of PTC-CFTR could be more effective than expected in vivo due to inflammatory conditions.

Funding: Cystic Fibrosis Foundation (GALIET22I0), Italian Cystic Fibrosis Foundation (FFC#4/2025, GMRF#1/2024), and Italian Ministry of Health (GR-2018-12367126).

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S 1.6 Correction of CFTR nonsense mutations by SRI-41315 alone or combined with Alyftrek

L. Damy[1], S. Mirval[1], C. Barrault[1], F. Becq[1]

[1]University of Poitiers, Laboratoire PRéTI, Poitiers, France

Therapeutic options for cystic fibrosis (CF) patients carrying CFTR nonsense mutations remain extremely limited. Our study aimed to evaluate the ability of the readthrough-inducing compound SRI-41315 to restore CFTR expression and function, alone or in combination with the vanzacaftor/tezacaftor/deutivacaftor regimen Alyftrek® .

We conducted a series of functional and biochemical assays using human bronchial epithelial cells from patients homozygous for F508del or compound heterozygous (F508del/R1162X, F508del/W1282X, G542X/E585X), CFBE41o cells expressing F508del-CFTR and FRT cells harboring nonsense variants (Y122X, G542X, R553X, W1282X). Cells were treated with SRI-41315 (1–3 µM), Alyftrek or both. CFTR activity was assessed using short-circuit current (Isc) measurements and whole-cell patch-clamp recordings. Protein maturation was analyzed by Western blot.

Our results show that treatment with SRI-41315 alone restored detectable CFTR-dependent chloride currents across all nonsense variants tested, demonstrating forskolin and ivacaftor/deutivacaftor responsiveness and complete inhibition by CFTRinh-172. Co-treatment with SRI-41315 and Alyftrek further increased current amplitude and promoted the appearance of the mature, fully glycosylated C-band on Western blots, indicating enhanced CFTR trafficking once full-length protein was produced by readthrough.

In conclusion, our findings identify SRI-41315 as a promising standalone or adjunct therapy to extend modulator-based therapy to class I CFTR mutations, addressing a major unmet need in cystic fibrosis treatment.

This work was supported by the french associations Vaincre la Mucoviscidose and Blanche through the DiTCAP program

S 2.1 Challenges and progress in the delivery of gene editors to airway epithelial cells

Katarina Kulhankova, Ashley L. Cooney, Brian C. Lewandowski, Yong Hong Chen, Colin Hemez, Alex A. Sousa, Shakayla Lamer, Greg A. Newby, Brian C. Lewandowski, Griffin Boysen, David R. Liu, David Guay, Anna X. Cheng, Patrick L. Sinn, Beverly L. Davidson, Paul B. McCray, Jr.

Department of Pediatrics, University of Iowa; Feldan Therapeutics;
Broad Institute of MIT and Harvard; Johns Hopkins University; Children's Hospital of Philadelphia.

CFTR modulators have transformed care for most people with CF (pwCF), but ~10-20% of pwCF harbor non-druggable mutations or are intolerant of modulators, underscoring the need for alternative therapeutic strategies. While long term expression may be obtained by CFTR gene addition, recent advances in gene editing technologies including base editing and prime editing provide opportunities to repair *CFTR* mutations at the genome level. A remaining significant hurdle to fully enable these tools for CF lung and other organ disease applications is effective delivery to target airway cell types, including progenitors.
Here we advance three approaches: peptide-mediated adenine base editor (ABE) RNP delivery, novel peptide modified AAV capsids, and helper-dependent adenoviral vectors. We delivered the ABE8e RNPs using an amphiphilic peptide and corrected sufficient CFTR R553X to restore anion transport. Using unbiased peptide-modified AAV libraries and round-over-round screening in well-differentiated primary cultures of human CF and non-CF airway epithelia (HAE), we identified 20 novel capsids that efficiently transduced cells at doses 10- to 100-fold lower than those required by existing vectors. We used these peptide-modified AAVs to 1) deliver a CFTR cDNA cassette to primary airway epithelia, and 2) edit the primary G551D locus using a single AAV ABE in primary CF pig airway epithelia. These approaches achieved restoration of CFTR function by electrophysiology. We previously optimized prime editing components for correcting the F508del mutation and demonstrated efficacy by delivery with electroporation. To advance this work, we packaged the required components in an all-in-one helper-dependent adenovirus (HDAd) vector. For proof of principle, we first applied this vector to the homozygous F508del cell line CuFi8. We achieved dose-dependent editing of the F508del locus and also achieved dose-dependent increases in CFTR-dependent anion current, similar to ETI. Vector formulation in hypertonic saline provided further increases in delivery efficiency for select AAVs and HDAd. Importantly, these delivery approaches facilitated access to basal progenitor cells. These results offer strategies to achieve delivery of CFTR expression cassettes and genome editors to target airway epithelial cells.

S 2.2 Highly efficent replacement of the *CFTR* cDNA to treat all people with cystic fibrosis

Sriram Vaidyanathan

Center for Gene Therapy. Nationwide Children's Hospital

Previous research by us and others has shown that diverse CFTR mutations spread throughout the gene cause CF in different populations. Therefore, precise replacement of the *CFTR* gene is necessary to treat all people with cystic fibrosis (pwCF). Gene replacement can be durable if achieved in airway basal stem cells (ABCs). CRISPR-Cas9 enables precise gene insertion and it requires the delivery of a template along with Cas9. Templates delivered using adeno-associated viruses have been the most effective. Replacement of the *CFTR* cDNA is challenging because it is a long gene (~4500 bp) that does not fit into the commonly used adeno-associated virus (AAV) vectors.

To optimize *CFTR* cDNA insertion in ABCs, we evaluate the use of non-viral vectors and AAV. Apart from single and dual AAV systems, we also tested linear and circular single stranded DNA to optimize gene insertion. Single AAVs were successfully packaged with the *CFTR* cDNA and very short homology arms corresponding to the *CFTR* locus. In combination with DNA repair inhibitors, this system achieved gene insertion in ~30% of alleles in primary ABCs from 3 different donors with CF. Upon differentiation, we achieved restoration of CFTR function that was 30-50% of non-CF controls. We also tested gene insertion using a sequential gene insertion system containing the two halves of the *CFTR* cDNA in two AAVs.

In combination with DNA repair inhibitors, this system achieved *CFTR* cDNA insertion in 40-60% of treated ABCs. We edited CF ABCs from 8 donors. Upon differentiation, we observed CFTR function that was comparable to no-CF controls. These efforts lay the foundation for the further development of gene edited autologous airway stem cell therapies to treat CF.

S 2.3 Virus-like particles for 'hit and go' gene editing to treat the cystic fibrosis gene defect

L.L. Dipalo[1,7], M. Bulcaen[1,7], P. Kortleven[1,7], K. Gryspeert[1], J. Van Slambrouck[1,2], A. Barbarossa[1,2], S. Thierie[1], M. Smits[3], M.M. Ensinck[1], G. Maule[4], A.S. Ramalho[5], F. Vermeulen[5,6], A. Cereseto[4], R. Gijsbers[3], L. J. Ceulemans[1,2], M.S. Carlon[1,8]

[1]Laboratory of Respiratory Diseases and Thoracic Surgery (BREATHE), Department of Chronic Diseases and Metabolism (CHROMETA), KU Leuven, Leuven, Belgium, [2]Department of Thoracic Surgery, University Hospitals Leuven, Leuven, Belgium, [3]Laboratory of Disease Modelling, Targeted Drug Discovery, and Gene Therapy (ADVANTAGE), Department of Pharmaceutical and Pharmacological Sciences, KU Leuven, Leuven, Belgium, [4]Department of Cellular Computational Integrative Biology (CIBIO), University of Trento, Italy, [5]Woman and Child unit, Department of Development and Regeneration, KU Leuven, Leuven, Belgium, [6]Department of Pediatrics, UZ Leuven, Leuven, Belgium, [7]These authors contributed equally, [8]Presenting author

Previous research by us and others has shown that diverse CFTR mutations spread throughout the gene cause CF in different populations. Therefore, precise replacement of the *CFTR* gene is necessary to treat all people with cystic fibrosis (pwCF). Gene replacement can be durable if achieved in airway basal stem cells (ABCs). CRISPR-Cas9 enables precise gene insertion and it requires the delivery of a template along with Cas9. Templates delivered using adeno-associated viruses have been the most effective. Replacement of the *CFTR* cDNA is challenging because it is a long gene (~4500 bp) that does not fit into the commonly used adeno-associated virus (AAV) vectors.

We applied base and prime editing to treat drug-refractory *CFTR* variants causing cystic fibrosis (CF), following a preclinical pipeline that utilizes models of increasing complexity, including patient-derived rectal organoids and differentiated airway cultures. Off-target (OT) edits were assessed using Guide-Seq and targeted deep sequencing, revealing no detectable OTs. To enhance translational delivery, we employed murine leukemia virus (MLV) based virus-like particles (VLPs), achieving rapid and efficient editing in various primary cell models.

While patient-derived in vitro models are invaluable to estimate gene editing efficiencies on- and off-target, considering aspects such as genomic context and DNA repair responses, these models fail to mimic translational delivery routes.

To address this outstanding question, we are developing a rat ex vivo lung perfusion (EVLP) model to support airway or systemic delivery of gene therapies. EVLP allows real-time monitoring of lung physiology, such as pulmonary compliance, thereby informing on the safety profile of a given delivery vehicle. VLP-fluc systemic delivery and recirculation during 6 hours on EVLP, did not elicit any change in compliance, nor induce any changes in pro-inflammatory cytokines compared to vehicle control, while promoting rapid fluc signal. Upon VLP-SpCas9 delivery targeting -proof-of-concept- the CXCL1 locus, genome editing was obtained in lung cells covering epithelial, endothelial and immune cells. Further studies are ongoing with increased doses to assess on- and off-target editing profiles, besides assessing the phenotypic consequences of the obtained gene editing. We intend to further expand this EVLP model for translational delivery and gene editing assessment for lung diseases, such as CF, and beyond.

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S 2.4 Silencing the silencers: antisense oligonucleotides targeting intronic splicing silencer motifs in CFTR

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Introduction: Among the ~2,100 reported potentially disease-causing CFTR variants, over 10% disrupt pre-mRNA splicing [1]. Yet the regulatory architecture driving these events - especially intronic splicing silencers (ISSs) and their RNA-binding protein (RBP) binding sites - remains poorly defined. Steric-blocking ASOs can restore exon inclusion by preventing repressive RBP-RNA interactions [2]. Our previous rescue of c.2657+5G>A by targeting putative downstream intronic elements suggested ISS interference as the mechanism, but the responsible motif was unknown [3]. Here, we map ISS motifs across CFTR and assess their value as therapeutic entry points for loss-of-splicing (LoS) variants.

Methods and results: We compiled a unified ISS motif set by integrating SpliceAid-F predictions with experimentally validated intronic silencers from the literature. Using this library, we mapped candidate and validated ISS motifs across the CFTR locus. We next assessed 328 CFTR single nucleotide variants with SpliceAI and MaxEntScan to predict LoS variant effects and scored each variant for integrity of its donor/acceptor site. Combining these metrics, we developed a prioritisation framework for the identification of variants most likely to respond to an ISS-blocking ASO strategy. This classified 141 variants as LoS and highlighted 50 with the highest predicted amenability - the highest ranked of which was the recurrent c.2657+5G>A. Local motif mapping around the intron 16 5' splice site revealed five experimentally validated ISS motifs in proximity to this variant. To test functional relevance, we co-transfected HEK293T cells with a CFTR c.2657+5G>A mini-gene and ASOs targeting the ISS motifs. Here we present data on restoration of exon 16 inclusion by these ASOs, and the extent to which steric inhibition of this site can rescue the splice defect associated with c.2657+5G>A.

Conclusions: We provide the first integrated map of intronic splicing silencer motifs across CFTR and identify a subset of LoS variants with therapeutic potential for ASOs blocking nearby ISS elements. Mechanistic validation in c.2657+5G>A mini-gene model suggests that targeting motif-rich regions can restore exon inclusion. Further motif-level mutagenesis will be used to refine this mechanism; however our preliminary evidence supports ISS-blockade as a viable correction strategy.

Acknowledgements: Work supported by UID/04046/2025 – BioISI centre grant from FCT, Portugal and ORGESTRA Doctoral Network (HORIZON-MSCA-2022- DN-01-01-101120108) from EU.

References: [1] Cystic Fibrosis Mutation Database, [2] Gao Y et al. Nucleic Acids Res. 2022;50(2):731–49. [3] Igreja S et al. Hum Mutat. 2016;37:209–15.

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S 2.5 Functionalized nonsense suppressor tRNA picovectors represent a novel therapeutic cargo for the treatment of PTC-associated CF

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Background: 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). For people with CF (pwCF), ~10% harbor CFTR a variant that could be responsive to therapies targeting the PTC codon, for which current CFTR modulators are ineffective. We have recently demonstrated the function of a series of nonsense suppressor anticodon edited tRNAs (ACE-tRNAs) to suppress CF-causing PTCs in the cystic fibrosis transmembrane regulator (CFTR) gene in gene-edited immortalized human bronchial epithelial (16HBE14ge) cells. 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 impart several favorable characteristics to the vectors including 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 produce a robust PTC suppression response.

Methods: 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 hairpins serving to covalently close the sLPV ends. Further, these hairpins were synthesized to contain functionalized nucleotides for click chemistry labeling with azide functionalized moieties. Introduction of this chemical functionality allows for dual labeling of the sLPVs with any combination of azide functionalized fluorophores, peptides, proteins, or other DNAs to improve either cellular delivery, intracellular trafficking, or tracking 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. To test the NLS-functionalized sLPV in a manner consistent with delivery to post-mitotic cells, aphidicolin was utilized to arrest cell division in 16HBEge cells.

Results and Conclusions: We tested several NLS sequences, with a scrambled NLS peptide serving as a control. All NLSs improved PTC suppression in aphidicolin treated 16HBEge cells. While the presence of dsDNA in the cytoplasm of human cells can trigger the cGAS-STING innate immune pathway, sLPV labeled with NLS produces ~80% less cGAMP as compared to plasmid DNA. This novel modular sLPV assembly procedure allows for production of minimal ACE-tRNA therapeutic vectors with properties properly tuned for optimal cellular delivery and intracellular trafficking.

Acknowledgements: Funding for this work was provided by a Cystic Fibrosis Foundation and NIH grants to JDL (LUECK20GO, and NHLBI 1 R01 HL153988-01A1 respectively).

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S 2.6 Engineered virus-like particles successfully deliver adenine-base editing to restore CFTR function by correction of R553X

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Premature termination codons (PTCs) in the CFTR gene present a major unmet therapeutic need in cystic fibrosis (CF), as individuals with Class I nonsense mutations such as R553X do not respond to current modulator therapies. Adenine base editing, a CRISPR-based technology that allows the transition of A-T base pairs into G-C base pairs in a targeted manner, offers a strategy to directly and precisely restore sense codons and CFTR expression. A major challenge is delivery of base editors in vivo in a safe and efficient manner. Engineered virus-like particles (eVLPs) offer a transient, non-integrating method for delivering base editor ribonucleoproteins directly to airway epithelial cells.

Vesicular stomatitis virus glycoprotein (VSV-G)-pseudotyped eVLPs were employed to deliver ABE8e with an R553X-specific sgRNA into 16HBEge-R553X cells. Gene editing outcomes were quantified by PCR and next-generation sequencing, and CFTR protein restoration was assessed by Western blot analysis. BE-eVLP transduction achieved dose-dependent correction of R553X, up to ~93% correction of R553X alleles, accompanied by recovery of CFTR protein to ~80% of wild-type expression. Edited cells maintained stable correction over three months, with corrected alleles increasing to ~99% over time, suggesting a proliferative advantage conferred by restored CFTR function.

We evaluated editing in primary nasal epithelial cells from R553X reporter mice harboring an Aka-Luciferase–mScarlet reporter construct containing the human R553X sequence. Highly efficient editing was achieved when cells were transduced with VSV-G-pseudotyped eVLPs, while in suspension, coincident with seeding. With established, confluent and polarised monolayers, basolateral-transduction resulted in many fluorescent and edited cells, but not apical-transduction. Estimates from reporter luminescence indicate basolateral transduction was 81-times more potent at delivering editing than apical.

Functional rescue of CFTR was tested by electrophysiology on 5-week-old polarised monolayers of primary nasal epithelial cells from humanised CFTR R553X/R553X mice. Ussing chamber assays demonstrated near wild-type levels of CFTR-mediated ion transport when transduction with VSV-G-BE-eVLPs (15 µl) was coincident with seeding onto filters. Short-circuit current measurements post-forskolin-treatment were: 4 µA for untreated verses 78 µA for transduced. Monolayers transduced apically, 2 days post-seeding, with 15 µl VSV-G-BE-eVLPs, registered a short-circuit current of 20 µA after 5 weeks in culture.

Robust and durable repair of the R553X CFTR mutation was achieved with substantial restoration of CFTR function in human and primary airway epithelial cells. VSV-G-pseudotyped eVLPs are most efficient at transducing polarised airway epithelial cells from the basolateral surface.

Given that PTC mutations lack effective treatments, BE-eVLP-delivered base editing represents a promising therapeutic strategy addressing an important unmet clinical need in CF. Future work will address pseudotyping of eVLP platforms to restore therapeutically meaningful CFTR function by targeting specific cell types in the lung and intestine; taking into account delivery route-requirements for polarised cell-surface targeting.

12 March - 14:15 - 16:00

Symposium 3
Inter-organ Crosstalk & Physiology

S 3.1 Lipid metabolism dysfunction in CF via the gut-liver axis

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In the Highly Effective Modulator Therapy (HEMT) era, there is an extension of life and health span. However, pwCF still experience persistent gastrointestinal dysfunction and trouble with dietary lipid absorption and BMI maintenance. In healthy individuals, dietary lipid absorption and the subsequent metabolism of dietary fat require the tandem efforts of the liver and the small intestine. A few of these key events include the hepatic production of bile acids, which are required in the small intestinal lumen to emulsify dietary lipids; hepatic first-pass detoxification and metabolism of intestinal effluent; the intestinal epithelial barrier which keeps bacteria, toxins, and contaminants out of the portal circulation while efficiently absorbing dietary lipids. Both tissues also synthesize and secrete specialized lipoproteins to deliver fat and cholesterol to the rest of the body.

In people with CF, there are several crucial defects in the gut-liver axis including: (1) fibrosis within the biliary tree and gallbladder that impinges bile acid secretion into the small intestine; (2) altered flow through the portal circulation which may impair hepatic detoxification, first-pass metabolism, and lipid metabolism; (3) the production of an altered bile acid pool (by both the liver and intestinal microbiota); and (4) impaired dietary lipid absorption by the intestine blood. These events significantly alter whole body metabolism and may contribute to poor GI outcomes in pwCF on ETI therapy.

This talk will cover the following:

(1) What is a healthy gut-liver axis, and what are the key clinical indices of dysfunctional gut-liver crosstalk ?
(2) How do the liver and the intestine maintain whole body lipid metabolism and how is this measured ?
(3) What are the major intestinal and liver phenotypes in pwCF and are these well understood ?
(4) How do we think lipid metabolism defects may impact other aspects of CF disease (i.e. lung function and infection, intestinal microbiota, metabolic disease, BMI) ?
(5) How does HEMT impact these events ?

S 3.2 Does the gut-lung axis play a role in cystic fibrosis disease?

Lucas Hoffman

Professor, Pediatrics (Pulmonary Division), Adjunct Professor, Microbiology, University of Washington, Seattle, USA

The term “gut-lung axis” describes an emerging concept: That there is cross-talk between the mucosal microbiology in one of those two organs (usually the gut) and the health of the other (usually the lung). Outside of cystic fibrosis (CF), there is growing evidence that gastrointestinal (GI) microbiota are altered in people with diverse lung diseases compared with healthy people, and that these relationships are attributable to cross-organ immune regulation, where the gut microbiota regulate immune activity in the lung. The evidence that a gut-lung axis contributes to disease pathogenesis in people with CF has expanded greatly in recent years; for example, some studies have found that microbes found in fecal samples are found later in respiratory samples from the same children, suggesting the GI tract can be a reservoir for respiratory infections. Elsewhere, several studies showed that fecal microbiomes correlate with measures of lung growth and function, and that fecal microbiology can indicate risk of respiratory exacerbations. These findings provide strong evidence that GI microbiology can influence respiratory disease in people with CF. Work in related diseases also suggests the possibility of a bidirectional relationship between CF GI and respiratory tracts—for example, that respiratory microbiology or inflammation could impact GI health and microbiomes—a concept that has been comparatively understudied topic in CF. In this talk, I will review the evidence that GI microbiota in people with CF impacts their respiratory tracts in clinically-relevant ways, highlighting potential mechanisms for these relationships and the evidence supporting those concepts. I will also underscore areas of opportunity—to better define the relationships between these two organs, the evidence for further effects of gut-lung interactions on other organs and systems (such as the liver and the endocrine system), the effects of current therapies such as antibiotics and CFTR modulators, and new avenues for disease modification suggested by these findings.

S 3.3 Extracellular vesicles in chronic muco-obstructive pulmonary diseases: role in pathogenesis and as biomarkers

Massimo Conese

Department of Clinical and Experimental Medicine, University of Foggia, Foggia, Italy

Muco-obstructive pulmonary diseases, such as chronic obstructive pulmonary disease (COPD) and cystic fibrosis (CF), are characterized by the accumulation of highly viscoelastic mucus in bronchi/bronchioles that compromises mucociliary clearance and fosters lung infection and inflammation, leading to a dysregulated airway regeneration and wound repair. Although CF and COPD stem from a different origin, one genetic and the other acquired, they share a similar pathophysiology, being the CF transmembrane conductance regulator (CFTR) protein implied in both disorders. Animal and plant cells secrete or shed small vesicles from their membrane called extracellular vesicles (EVs), functioning as a long distance cell-to-cell communication mechanism. Various subsets of EVs, comprised mainly of microvesicles (MVs) and exosomes (EXOs), are secreted by various cell types that are either resident or recruited to the airways during the onset and progression of CF and COPD lung disease, representing a vehicle for metabolites, proteins and RNAs, that in turn lead to events such as neutrophil influx, the overwhelming of proteases (elastase, metalloproteases), oxidative stress, myofibroblast activation and collagen deposition. Eventually, all of these pathomechanisms lead to chronic inflammation, mucus overproduction, remodeling of the airways, and fibrosis, thus operating a complex interplay among cells and tissues. The detection of MVs and EXOs in blood and biological fluids coming from the airways (bronchoalveolar lavage fluid and sputum) allows the consideration of EVs and their cargoes (especially microRNAs) as promising biomarkers for CF and COPD, although clinical expectations have yet to be fulfilled. Plant-derived nanovesicles (PDNV), especially EXO-like ones, display fruitful properties, similar to the mammalian EXO, with the advantages of simplicity, safety, eco-friendliness, low cost, abundant resources, and low immunogenicity in vivo. Recent studies indicate the potential therapeutic usefulness of PDNV in the treatment of wounds and thus their application to muco-obstructive pulmonary diseases.

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S 3.4 Cystic fibrosis airway epithelium secretome profiling: new insights into CFTR-related dysregulations

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The airway epithelium contributes to host defense through a variety of mechanisms, including the secretion of a wide range of defense proteins and peptides, antioxidants, anti-proteases, growth factors, chemokines and cytokines. In cystic fibrosis (CF), there is a vicious circle of respiratory infection, exaggerated neutrophil-predominant lung inflammation, epithelial remodeling and lesions favoring mucus stasis and respiratory infection. Lung lesions appear to be largely the consequence of a failure of airway epithelial defense. It has also been suggested that airway inflammation is due to epithelial dysfunction secondary to CFTR mutations, resulting in impaired innate host defense systems. However, the link between altered innate host defense proteins in CF surface liquid and CFTR defects has not been clearly demonstrated.

Our aim was to assess the impact of the CFTR defect on the secretome of CF bronchial epithelium, without contamination by inflammatory cells and bacterial secretions, while retaining the influence of inter-patient variability and directional epithelial secretion associated with polarization and differentiation.

Bronchial epithelial cells from CF patients (F508del/F508del; n=8) and healthy subjects (lung donors; n=8) were cultured at the air-liquid interface for 35 days until complete epithelial differentiation. The 24-hour secretions from these epithelia were collected and analyzed by quantitative liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS).

Proteome profiling identified and quantified 1629 proteins, of which 118 were found differentially abundant between non-CF and CF secretions (log 2 fold change $\geq$ 0.58; $p<0.05$), with 29 proteins upregulated and 89 proteins downregulated in CF secretions. Ninety-four differentially expressed proteins (80%) were classified as secreted according to the SecretomP and SignalP databases and the Human Secretome Atlas initiative. Seventy-three biological processes (Gene Ontology) were enriched. According to the Reactome database, 18 signaling pathways were enriched in differentially identified proteins, among which immune system pathways are the most enriched and represented, together with protease/antiprotease activity.

Our results show a CFTR-related dysregulation of the expression and secretion of numerous proteins, notably involved in host defense. These observations are indicative of a CF airway epithelium with a constitutive altered innate immunity, suggesting that the downstream consequences of CFTR mutation set the stage for chronic inflammation and infection in the cystic fibrosis airways.

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S 3.5 Cystic fibrosis associated colorectal cancer: linking CFTR-deficiency to the increased risk of developing CRC

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Background: Life expectancy in people with cystic fibrosis (pwCF) has significantly improved because of advances in clinical care and the introduction of CFTR modulators. However, recent studies indicate that ageing pwCF experience age-associated comorbidities, including an increased risk of developing colorectal cancer (CRC). PwCF aged 40-49 show a CRC prevalence of ~23%, compared to 2-5% in similarly aged non-CF individuals.

Understanding how CFTR deficiency affects intestinal epithelial biology, particularly its potential tumor-suppressive roles, is essential for improving CRC prevention and treatment in this population.

Therefore, we aim to investigate cell-autonomous mechanisms involved in CFTR loss-of-function (LoF) for the development of CRC. In parallel, we characterized polyp incidence in the CF population and established a biorepository of polyps from CF and non-CF patients to validate mechanistic findings in clinically relevant tissue.

Methods: We exploited previously generated non-isogenic patient-derived intestinal organoid (PDIO) cultures across a range of CFTR genotypes and generated CRISPR-engineered isogenic CFTR-KO organoid pairs. Proliferation and growth factor dependency were quantified under varying Wnt conditions. Additionally, PDIOs homozygous for F508del were treated with or without CFTR modulators (Elexacaftor/Tezacaftor/Ivacaftor). To identify pathways altered by CFTR-LoF in an unbiased manner, we performed bulk RNA sequencing followed by ongoing validation using qPCR, western blotting, and Luminex.

Clinical data from pwCF treated at the UMCU were analyzed for polyp incidence, dysplasia type, age, and modulator use. A biorepository of CF and non-CF polyps obtained during colonoscopy was established and will be used for spatial transcriptomics.

Results: In complete organoid medium, CFTR-KO organoids displayed reduced growth relative to its isogenic wild type. Under reduced Wnt conditions, however, CFTR-KO organoids demonstrated a proliferative advantage, revealing diminished Wnt dependency as a direct result of CFTR-LoF. In F508del/F508del PDIOs, ETI treatment enhanced growth compared with untreated cultures. In reduced Wnt conditions, untreated F508del organoids displayed higher proliferation, mirroring the phenotypes observed in isogenic CFTR-KO models. These findings indicate that CFTR activity could influence epithelial growth control and Wnt pathway sensitivity. Bulk RNA sequencing revealed reduced expression of the Wnt inhibitor DKK1 under CFTR-LoF conditions, providing a potential mechanistic link between CFTR deficiency, altered Wnt signaling dynamics, and increased proliferative capacity.

Clinical cohort analysis is expected to be finalized in January 2026.

Conclusion: This work establishes a mechanistic framework connecting CFTR-LoF to CRC susceptibility and highlights epithelial organoids as a powerful system to explore CFTR-dependent tumor-suppressive pathways. The complementary clinical dataset and biorepository will further strengthen the translational impact of these findings.

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S 3.6 Neutrophil transmigration across cystic fibrosis bronchial epithelia is modulated by hyperglycemia

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Background: Lifelong, massive migration of neutrophils into the cystic fibrosis (CF) airway in response to chronic infection results in inflammation, which is worsened in CF-related diabetes (CFRD). We hypothesized that during transmigration to the airways, neutrophils interact with bronchial epithelial cells leading damage of the airway monolayer in a variety of ways.

Methods: We utilized our established bronchial epithelial neutrophil transmigration model based upon 16HBE and primary airway epithelial cells expressing WT-CFTR or F508del-CFTR (16HBE-WT, 16HBE-CF, NhBE, and CFhBE). We tested the possible impacts of neutrophil transmigration on the bronchial epithelial monolayer with confocal microscopy, ELISA for neutrophil-based enzymes, bulk RNA sequencing, and immunoblotting.

Results: We found that Matrix Metallopeptidase 9 (MMP9), Myeloperoxidase (MPO), and Neutrophil elastase (NE) were significantly higher in the post-transmigration media compared to control groups without neutrophil transmigration, in NhBE and CFhBE monolayers and was dependent upon glycemic state. In addition, bulk RNA sequencing of NhBE and CFhBE monolayers with and without neutrophil transmigration demonstrated many differentially expressed genes, including genes for gap junctions, tight junctions, glucose transporters, oxidative stress, matrix metallopeptidase, and more, were significantly altered in the groups conditioned to high glucose and with neutrophil transmigration. Interestingly, we also observed that CellTracker dye loaded into neutrophil cytoplasm was frequently exchanged into adjacent bronchial epithelial cells during transmigration, indicating functional interactions between neutrophils and epithelial cells. This phenomenon exhibited a time-dependent increase in frequency.

Conclusion: These data show that neutrophils functionally interact with bronchial epithelial cells during transmigration.

Keywords: Bronchial epithelial cells; neutrophils; transmigration; CFRD; bulk RNAseq

Symposium 4
Metabolic Dysfunction in CF

S 4.1 CFRD modifies the sputum microbiome: implications for HEMT

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Cystic fibrosis related diabetes (CFRD) affects 40-50% of adults with cystic fibrosis (CF) and is associated with a decline in respiratory health. CFRD is a complex disease, with endocrine, inflammatory and metabolic components but is characterised by elevations in blood glucose concentration and HaemoglobinA1c. In the lungs, elevation of blood glucose in CFRD leads to changes in lung epithelial barrier function, the concentration of glucose and metabolites in the lumen, the generation of advanced glycation end products (AGE), inflammatory responses and the innate immune function of the epithelium. *In vivo* and *in vitro*, elevation of blood/media glucose increases luminal abundance of inoculated respiratory pathogens such *S. aureus* and *P. aeruginosa* indicating that hyperglycaemia in CFRD contributes to increased respiratory infection and pulmonary decline.

While the resident microbial flora of the lung has been shown to change with the development of CF disease, the effect of CFRD on the microbiome is less well understood. We therefore analysed the microbiome in sputa from 14 people with CF and 14 with CFRD and two who were classed as pre-CFRD, by extracting DNA and amplifying the variable V3-V4 region of the microbial 16S gene by PCR. Sequences were analysed and source identified to genus level. We found that the α-diversity of the microbiome was increased in CFRD compared to CF. Bray Curtis dissimilarity analysis indicated a separation of the microbiomes in CF and CFRD sputa. Like that reported in CF, the most abundant phyla identified in the sputum samples were *Firmicutes* and *Proteobacteria, Actinobacteriota* and *Bacteroidota* however, the ratio of *Firmicutes/Bacteroidota* was reduced in CFRD, reflecting that reported in the gut of people with diabetes. *Pseudomonas, Azhorizophilus, Porphyromonas* and *Actinobacillus* were more abundant in CFRD compared to CF. The relative abundance of these genera correlated well with HbA1c and less with a decline in FEV1/FVC indicating that development of hyperglycaemia in CFRD mediates further changes to the respiratory microbiome in CF. Further work is required to understand if highly effective modulator therapies (HEMT) for CF can improve glycaemic control, reverse CFRD-induced changes to the microbiome and reduce respiratory decline.

S 4.2 Mitochondrial alterations that shape CF lung infection

Sebastián A. Riquelme

Columbia University Irving Medical Center, Department of Pediatrics, Division of Infectious Diseases

Mutations in the cystic fibrosis transmembrane conductance regulator (CFTR), the genetic basis of cystic fibrosis (CF), induce profound alterations in host cell mitochondrial function. However, how this disrupted bioenergetic landscape contributes to CF disease progression remains poorly understood. In this presentation, I will integrate multiple lines of evidence demonstrating how mitochondrial dysfunction shapes pulmonary infection by major CF pathogens, including Pseudomonas aeruginosa and Staphylococcus aureus, as well as the host immune trajectories that favor pathogen persistence through disease tolerance.

Drawing on both published and unpublished findings, I will highlight how CFTR dysfunction reprograms mitochondrial bioenergetics in epithelial and myeloid cells, thereby exacerbating P. aeruginosa and S. aureus–driven lung disease. I will describe key mitochondrial and metabolic pathways perturbed by CFTR mutations — including the metabolic checkpoint PTEN, the immunometabolite itaconate, the de novo pyrimidine synthesis pathway, and purinergic signaling — and discuss how this altered microenvironment promotes bacterial adaptation by driving a transition toward biofilm-centered lifestyles. I will further illustrate how these biofilm communities align with host defense programs to engage disease tolerance mechanisms, enabling long-term persistence through responses uncapable to resolving infections.

Finally, I will examine how this immunometabolic landscape is reshaped by CFTR modulator therapies and discuss how these pathways may be therapeutically exploited to control bacterial burden via both antibiotic-dependent and antibiotic-independent strategies.

Beyond addressing fundamental questions at the interface of CFTR function and mitochondrial bioenergetics, this work provides a conceptual framework for understanding how disruption of this equilibrium fosters a microenvironment permissive to chronic infection and progressive airway remodeling. Collectively, these findings position mitochondrial metabolism as an actionable target to modify pathological disease trajectories in CF, including persistent infection and lung deterioration.

S 4.3 Early life pancreatic remodeling in CF ferrets

John F. Engelhardt

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Cystic fibrosis (CF) profoundly disrupts pancreatic maturation beginning during late fetal development and after birth, yet the mechanisms linking early exocrine injury, ductal remodelling, and endocrine dysfunction remain poorly understood. Using the CFTR-knockout and G551D ferrets, an animal model that closely mirrors human CF pancreatic disease, we characterized a sequence of early-life remodelling events that may influence progression to CF-related diabetes (CFRD). CF ferrets experience distinct glycemic phases, including a pronounced hyperglycemic crisis at ~ 1 month of age followed by a transient glycemic recovery, coinciding with dynamic shifts in pancreatic inflammation, fibrosis, adipogenesis, and islet regeneration. Early exocrine destruction coincided with loss of pancreatic endocrine cells, followed by a surge in pancreatic PDX1 expression that correlated with re-emergence of islet structures and partial re-establishment of glucose homeostasis. To examine CF-associated changes in the ductal epithelium and their potential impact on islet function in the absence of the exocrine pancreas, we developed methods to propagate and polarize ferret pancreatic ductal epithelium. Complementary proteomic, transcriptomic, epigenomic, and single-cell analyses revealed that loss of CTFR protein fundamentally reprograms the ductal epithelium. CF ductal cells exhibited aberrant activation of PDX1, suppression of PEN/GSK3β signaling, and induction of WNT and TGF-β pathways, promoting expansion of centroacinar and bipotent progenitor-like populations. These altered epithelial states adopted features resembling acinar-to-ductal metaplasia and pancreatic developmental immediates Using lineage-tracing transgenic ferrets, we are investigating wether early-life developmental programs in the CF pancreas support islet regeneration from reprogrammed ductal progenitors. Together, these findings establish a model in which CFTR deficiency initiates early pancreatic injury, triggers ductal and progenitor-cell remodeling, and shapes a phasic pattern of endocrine dysfunction in CF. The altered ductal phenotype depends on the presence of CFTR protein, but not its channel function, and is therefore relevant to CFTR mutations that produce little or no protein product. Understanding these developmental transitions in CF ferrets provides mechanistic insight into the early metabolic instability observed in young children with CF and highlights potential therapeutic targets for the treatment and prevention of CFRD.

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S 4.4 Metabolic regulation of pulmonary infection trajectory in cystic fibrosis

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Background: Bacterial infection profoundly accelerates pulmonary decline in people with cystic fibrosis (pwCF). S. aureus (SA) typically predominates during early childhood, whereas P. aeruginosa (PA) emerges later and further entrenches the maladaptive inflammatory environment of the CF airway. Yet the mechanisms underlying this characteristic infection trajectory remain poorly defined. Here, we investigated how the airway metabolic landscape shaped by CFTR mutations, together with the adaptive capacity of CF pathogens, contributes to the progression from SA to PA co-infection, with a particular focus on sulfur-centered metabolic networks.

Aims: This study aimed to determine how CFTR-driven metabolic alterations orchestrate infection trajectories through shared host–pathogen metabolic adaptations.

Methods: Airway metabolic alterations associated with CFTR mutations were examined by LC-MS metabolomics of sputum and airway cell supernatants from CF and non-CF donors. To identify adaptive mutations, we performed whole-genome sequencing (WGS) on SA isolates from CF (n=560) respiratory samples. Functional studies used the JE2 WT strain and its Tn::cysM transposon mutant, which is unable to convert L-homocysteine into L-cystathionine, L-cysteine, and taurine. Bacterial growth was assessed in LB and artificial sputum medium (ASM) supplemented or not with sulfur-rich metabolites (L-methionine, L-homocysteine, L-cystathionine, L-cysteine, taurine). Infection outcomes — including bacterial burden (CFU enumeration) and secretion of CF-relevant cytokines (ELISA) — were evaluated in non-CF and CF airway epithelial (F508del/W1282X) and differentiated myeloid cells (F508del/F508del) using gentamicin protection assays. In vivo relevance was assessed using a mouse model of respiratory infection.

Results: CF sputum and supernatants from CF airway epithelial cells were enriched in sulfur-containing metabolites, including L-methionine, N-acetyl- methionine, L-cystathionine, taurine, and S-cysteinosuccinate. WGS revealed that ~78% of SA isolates from pwCF harbor inactivating mutations in cysM, a gene essential for the generation of cysteine through the conversion of L-homocysteine into L-cystathionine. Functional studies in LB demonstrated that cysM inactivation confers resistance to the stress imposed by sulfur metabolites like cysteine. ASM provided similar protection to cysM-intact strains, supporting an adaptive convergence between bacterial genetics and the CF metabolic milieu. In human CF epithelial cells, cysM inactivation enhanced IL-8 secretion.

Although this mutation did not increase SA persistence in neutrophils, during co-infection with PA it markedly promoted PA survival — an effect reproduced in vivo.

Conclusion: These findings reveal that the sulfur-rich airway environment generated by CFTR dysfunction imposes selective metabolic pressure favoring cysM-deficient SA strains. These organisms are more resilient to the CF metabolic milieu, elicit heightened epithelial inflammation, and — critically — facilitate PA persistence. Together, our data provide a metabolic explanation for the characteristic polymicrobial infection trajectory in CF and identify sulfur metabolism as a key driver of pathogen evolution and community dynamics in the CF airway.

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S 4.5 A secretory cell is never late: CFTR emerges early in epithelial regeneration

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Introduction: In people with cystic fibrosis (pwCF), chronic infection and inflammation lead to repeated cycles of airway epithelial injury and repair. Current knowledge of CFTR function comes from mature, well-differentiated airway epithelial cultures, where ionocytes and secretory cells show the highest CFTR activity. However, epithelial regeneration is dominated by transitional secretory cells, and whether these early cell states contribute to CFTR function remains unknown. We therefore examined CFTR expression and activity during early differentiation to determine whether transitional secretory-lineage cells play a functional role.

Methods: Human nasal epithelial cells (HNECs) from non-CF subjects (n=3) were differentiated in air-liquid interface (ALI) cultures. Mucociliary differentiation was characterized through immunofluorescence and qPCR for secretory and ciliated markers on days 0, 7, 14, and 21. At the same time points CFTR-dependent chloride transport was assessed using Ussing chamber recordings. CFTR function during early differentiation was furthermore evaluated in day-7 monolayer-derived airway organoids from pwCF (F508/delF508del; n=2) using the Forskolin induced swelling (FIS) assay. Finally, CFTR modulator (ETI) responses were quantified using the FIS assay in secretory-enriched vs ciliated-enriched organoids, representing early vs late differentiation states, respectively.

Results: Investigation of mucociliary kinetics revealed a significant increase in MUC5AC+ secretory cells at day 7, followed by a decline at day 14 and 21, coinciding with an increase in β-tubulin IV+ ciliated cells at day 14 and 21. qPCR analyses of MUC5AC, CFTR, and the goblet-cell regulator SPDEF supported these observations. In addition, CFTR-dependent ion transport in Ussing chambers showed a comparable temporal profile. Organoids from early differentiated airway monolayers of pwCF formed cystic structures containing MUC5AC+ cells and displayed ETI responses in FIS assays. Furthermore, ETI responses were significantly higher in secretory-cell-enriched organoids compared to ciliated-cell–enriched organoids, consistent with the early secretory dominance during regeneration.

Conclusion: We show the early regenerative airway epithelia rapidly adopt a secretory-dominant state that transitions towards ciliated cells over time.

These transitional-secretory airway epithelia display high CFTR function that declines as differentiation progresses, suggesting a previously unrecognized role for early secretory lineages in epithelial regeneration and possibly CF-related repair defects

P 7

S 4.6 Targeting a PI3Kγ–AKAP13–PKD1 signaling axis enhances F508del-CFTR trafficking and potentiates ETI therapy

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Background: Elexacaftor/tezacaftor/ivacaftor (ETI) therapy, the current standard of care for individuals carrying the F508del-CFTR mutation, combines 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 substantially improves lung function and clinical outcomes in patients heterozygous for F508del, it only partially restores CFTR activity and stability. Consequently, patients continue to experience mucus dysfunction, airway infection, and inflammation, highlighting the need for complementary therapeutic strategies.

Aims: Because boosting cAMP/PKA signaling promotes plasma membrane (PM) CFTR stabilization and enhances CFTR modulator efficacy, we hypothesized that the cAMP-elevating peptide previously developed in our laboratory (PI3Kγ MP; Patent No. WO/2016/103176; Ghigo et al., Sci Transl Med., 2022) could further increase F508del-CFTR PM density and stability, thereby potentiating the therapeutic benefit of ETI.

Methods: Cell-surface protein biotinylation followed by western blotting and immunogold electron microscopy were employed to assess CFTR PM density in HEK293T, 16HBE14o-, and CFBE41o- cells treated with PI3Kγ MP. Primary human bronchial epithelial (HBE) cells were also used to evaluate the functional impact of the PI3Kγ MP + ETI combination. Phosphoproteomic profiling was performed to identify signaling pathways activated by PI3Kγ MP.

Results: Combining PI3Kγ MP with ETI maximized ETI's corrective efficacy in CFBE41o- cells expressing F508del-CFTR by increasing CFTR abundance at the PM compared with ETI alone. Following six hours of cycloheximide treatment, 30% of ETI-rescued F508del-CFTR remained undegraded, whereas 67% persisted when PI3Kγ MP was added, indicating that PI3Kγ MP doubles the stability of corrected F508del-CFTR. Phosphoproteomic analysis revealed marked activation of protein kinase D1 (PKD1), a key orchestrator of protein trafficking. Notably, inhibition of PKD1 abrogated the PI3Kγ MP–mediated stabilization of F508del-CFTR, identifying PKD1 as the central effector of this pathway. Moreover, PKD1 activation required coordinated PKA and PKC signaling through A-kinase anchoring protein 13 (AKAP13/AKAP-Lbc). Finally, to validate these findings in a more physiologically relevant context, the combination of PI3Kγ MP and ETI was tested in primary HBE cells derived from five independent CF donors (three F508del/F508del and two F508del/G542X), resulting in up to a 25% increase in CFTR activity compared with ETI alone, confirming its potential as an add-on therapy.

Conclusions: This study identifies PI3Kγ and PKD1 as pivotal regulators of CFTR stability at the plasma membrane and highlights AKAP13 as a novel contributor to CFTR signaling. By enhancing F508del-CFTR trafficking and potentiating the efficacy of ETI therapy, PI3Kγ MP emerges as a promising add-on strategy for cystic fibrosis. Importantly, this compound is currently under clinical evaluation and has successfully completed Phase I, underscoring its translational potential and readiness for further therapeutic development.

This work is supported by the Italian Cystic Fibrosis Research Foundation (FFC)

13 March - 11:00 - 12:45

Symposium 5
Vascular and Cardiovascular complications

S 5.1 CF community survey results

Paula Sommer

Cystic Fibrosis Trust
London, United Kingdom

No abstract submitted.

S 5.2 Cardiovascular Risk in Cystic Fibrosis in the Modulator Era: Emerging Evidence and Unanswered Questions

Prof Damian G. Downey

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

The sustained improvement in survival associated with CFTR modulation has shifted the clinical trajectory of cystic fibrosis (CF). As people with CF (pwCF) age, attention is increasingly turning toward long-term comorbidities, including cardiovascular disease (CVD). While absolute event rates remain relatively low, emerging epidemiological and mechanistic data suggest that cardiovascular risk in CF may be evolving in ways not previously anticipated.

Registry-linked and federated health database analyses indicate an increased relative risk of major adverse cardiac events compared with matched populations. Subclinical vascular abnormalities are detectable even in younger pwCF, including endothelial dysfunction, increased arterial stiffness, and mild ventricular impairment. Reduced left ventricular function has been associated with adverse outcomes, raising the possibility that cardiovascular vulnerability in CF is not solely a late consequence of advanced lung disease.

Multiple intersecting pathways may contribute. Chronic systemic inflammation, CF-related diabetes, insulin resistance, and changing lipid profiles create a pro-atherogenic environment. CFTR expression in vascular endothelium and cardiomyocytes suggests that intrinsic CFTR dysfunction may influence myocardial and vascular biology directly. Experimental data implicate oxidative stress, altered nitric oxide signalling, and endothelial activation as potential mediators.

The introduction of elexacaftor/tezacaftor/ivacaftor further complicates the cardiometabolic profile. Weight gain, rising blood pressure, and increasing prevalence of metabolic syndrome have been observed in subsets of treated individuals. Whether these represent adaptive nutritional restoration or early cardiometabolic transition requires clarification through longitudinal study.

The critical issue is not simply whether cardiovascular events occur in CF, but whether current clinical frameworks adequately characterise risk trajectories in an ageing population. This presentation will synthesise emerging evidence and outline a translational strategy integrating vascular phenotyping, imaging, biomarker profiling, and registry-linked outcome modelling to define cardiovascular risk across the lifespan in CF.

If gains in survival are to translate into healthy longevity, cardiovascular health must become a deliberate and measurable component of CF research and care.

S 5.3 Vascular changes in the end-stage CF lung – what is the link with CFTR?

Mieke Boon, MD PhD

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Pulmonary hypertension (PH) is an important, life-limiting co-morbidity in cystic fibrosis (CF). Multiple mechanisms such as local inflammation and hypoxia are believed to be the main drivers of increased vascular resistance. However, it is clearly demonstrated that CFTR is expressed by endothelial cells and vascular smooth muscle cells and therefore primary CFTR dysfunction may affect pulmonary vascular resistance and integrity. Although expression of CFTR in endothelium and the impact of its dysfunction on vascular smooth muscle constriction was already shown in CF as well as in idiopathic PH, it was unclear to what extent dimensions of pulmonary arterioles are affected.

Therefore, we used a 3D morphometric analysis of cores excised from end-stage explant CF lungs to perform evaluate distal pulmonary arterioles dimensions and their accompanying distal airways.

We found that distal pulmonary arteries were narrowed and even completely disappeared in the last generations of dichotomous branching, resulting in a decreased total diameter in the last generations. This process of vascular pruning is the correlate of increased pulmonary vascular resistance and is probably linked to the development of PH. On histology, fibrosis of the smooth muscle layer and thickening of the basement membrane, with disruption of the elastic membrane is seen.

Although vascular abnormalities seemed to be related to the severity of airway damage, a decreased diameter of distal arterioles was evenly seen in zones without airway damage. These findings suggest that vascular dysfunction in the CF lung may not solely be secondary to hypoxic vasoconstriction and inflammation but may represent a distinct pathophysiological process related to CFTR dysfunction in the endothelium and vascular smooth muscle cells of the pulmonary circulation.

Previous *in vitro* research from our group nicely showed that CFTR knock-out in endothelial cells induces a pro-inflammatory state of the endothelium, that could partially be rescued by CFTR modulator use *in vitro*. Thus, endothelial dysfunction, increased inflammation and altered smooth muscle tone are important players in the development of PH in CF.

From a therapeutic perspective, targeting vascular smooth muscle thickening (including anti-fibrotic therapy ?) may be relevant in patients with severe PH, as PH may worsen respiratory outcomes.

S 5.4 Imaging the Brain in People with Cystic Fibrosis

Dr Hannah Chandler (Speaker), Dr Jamie Duckers, Professor Richard Wise and Dr Claudia Metzler-Baddeley (Grant PI / co-authors)

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Cystic fibrosis (CF) is a progressive inherited disorder that historically limited life expectancy; however, advances in CFTR modulator therapies have revolutionised the lives of people with CF (PwCF). As life expectancy improves, PwCF are increasingly exposed to age-related comorbidities, including cardiovascular disease, stroke, and cognitive impairment, which may be occuring at an earlier age than expected. Despite established links between systemic vascular health and brain function, cerebrovascular physiology and cerebral oxygen metabolism have not been systematically examined in PwCF, limiting our ability to anticipate long-term neurological risk in this population.

In our initial proof-of-concept pilot study we scanned 14 PwCF and fifty-six healthy age-and sex-matched controls underwent magnetic resonance imaging (MRI) to assess brain volumes, cerebrovascular function and tissue oxygenation. Our initial results provide the first evidence of altered cerebral oxygen metabolism and region-specific perfusion changes in PwCF, despite preserved global cerebral blood flow. Elevated $CMRO^2$ may represent an early marker of increased metabolic demand or compensatory cerebrovascular stress in the context of chronic systemic inflammation, hypoxia, or accelerated biological aging. Clinically, these findings support the need to consider brain health as an emerging component fo long-term CF care. Non-invasive neuroimaging measures of cerebrovascular function may offer valuable tools for early risk stratification, longitudinal monitoring, and evaluation of therapeutic interventions aimed at preserving cognitive function and reducing future cerebrovascular risk in the adult CF population.

14 March - 08:45 - 10:30

Symposium 6
Organ-on-a-Chip & New Models

S 6.1 Organ-on-a-Chip Technology for Modeling Human Lung Pathophysiology

Roberto Plebani

Department of Medical, Oral and Biotechnological Sciences, G. d'Annunzio University of Chieti-Pescara - Italy

Cystic Fibrosis (CF), as well as other lung diseases, is characterized by persistent inflammation and recurrent infections. Chronic inflammatory states are closely associated with progressive tissue damage and can act as initiating factors for aberrant cellular remodeling and potential malignant transformation.
One of the major limitations in biomedical research is the paucity of predictive and physiologically relevant preclinical models. Conventional two-dimensional cell cultures and animal models fail to fully recapitulate the structural, cellular, and dynamic complexity of the human lung microenvironment. In recent years, the Organ-on-a-Chip (OoC) technology has emerged as a powerful platform to model human lung pathophysiology. These microfluidic, three-dimensional systems, composed of one or multiple interconnected compartments, integrate key aspects of tissue architecture, mechanical stimuli, and dynamic fluid flow, providing a more faithful representation of in vivo conditions compared to cell cultures.

In the context of CF and other chronic inflammatory lung diseases, OoC platforms enable quantitative and real-time investigation of inflammatory processes, including the recruitment and transmigration of polymorphonuclear neutrophils (PMNs), a defining hallmark of CF lung pathology. Beyond PMN recruitment, 3D and Organ-on-a-Chip models allow the investigation of broader cellular migration phenomena and complex multicellular interactions within physiologically relevant microenvironments. By combining multiple tissues and immune cells, these systems offer unique opportunities to monitor inflammation and testing drugs in a relevant preclinical model made of human cells.

Importantly, the applicability of the OoC technology extends beyond inflammatory lung diseases. Similar approaches can be leveraged to study pathological cell migration in other disease contexts, including cancer, where early migratory events and epithelial–mesenchymal transition play a pivotal role in tumor progression and metastasis.

This contribution will highlight how Organ-on-a-Chip technology can bridge critical gaps in preclinical modeling of human lung diseases, with a particular focus on CF-related inflammation, immune cell recruitment, pathogen infections, and cell migration. Moreover, the integration of iPSC-derived cellular models could open new avenues to further enhance the physiological relevance and translational value of these innovative culture systems.

S 6.2 The bronchioid model: a tool to recapitulate features of distal airways and disease-relevant phenotypes

Katharina Raasch[1,2,*], Yamaris Mena-Cabrera[1,2,*], Eloïse Latouille[1,2], Elise Maurat[1,2], Aurélien Richard[3], Pauline Henrot[1,2,4], Maeva Zysman[1,2,4], Vanessa Bergeron[3], Pauline Esteves[1,2], Hugues Bégueret[1,2,3], Matthieu Thumerel[1,2,3], Pierre Nassoy[5], Patrick Berger[1,2,3], Laetitia Andrique[3], Gaëlle Recher[5], Isabelle Dupin[1,2,6]

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Airflow limitation is the hallmark of obstructive pulmonary diseases, with the distal airways representing a major site of obstruction. Although numerous *in vitro* models of bronchi already exist, there is currently no culture system for obstructive diseases that reproduces the architecture and function of small airways. We developed a so-called bronchioid model by encapsulating human bronchial adult stem cells derived from clinical samples in a tubular scaffold made of alginate gel. This template drives the spontaneous self-organisation of epithelial cells into a tubular structure. Three-dimensional imaging, gene expression and single-cell RNA-sequencing analysis of bronchioids made of bronchial epithelial cells revealed tubular organisation, epithelial junction formation and differentiation into ciliated and goblet cells. Ciliary beating was observed, at a decreased frequency in bronchioids made of cells from patients with chronic obstructive pulmonary disease (COPD). The bronchioid could be infected by rhinovirus. An air–liquid interface was introduced that modulated gene expression. We are currently incorporating bronchial smooth muscle cells into the bronchioid model. Preliminary results indicate enhanced robustness of the bi-component bronchioid, as well as greater persistence of basal epithelial cells in the bi-compartment model compared with the mesenchyme-free version. Here, we provide a proof of concept of a perfusable bronchioid with proper mucociliary function. The key advantages of our approach, such as the air liquid interface, lumen accessibility, recapitulation of pathological features, possible assessment of clinically relevant end-points and the integration of the mesenchymal compartment, will make our pulmonary organoid-like model a powerful tool for understanding obstructive pulmonary diseases.

S 6.3 Complex airway models to study infection in CF

Helle Krogh Johansen[1,2,3], Pablo Laborda[1], Albert Fuglsang-Madsen[1], Alexander Melanson[1], Janus Haagensen[1], Claudia Antonella Colque[1], Ruggero La Rosa[2], Søren Molin[2].

Dept. Clinical Microbiology, Rigshospitalet, Copenhagen, Dept. Health Technology, The Technical University of Denmark, Kgs. Lyngby and Dept. Clinical Medicine, Faculty of Medical and Health Sciences, University of Copenhagen, Copenhagen, Denmark.

Background: Persistent bacterial infections pose significant clinical challenges and increase the use of last-resort antibiotics. In some people with CF, bacterial infections treated with antibiotics persist despite *in vitro* diagnosed antibiotic susceptibility, highlighting that standard laboratory conditions may fail to mimic the infected airway environment in a patient.

Methods: We have developed an air–liquid interface (ALI) airway epithelial model cultured either in Transwell vials or in micro-fluidic devices. Further, we have added stimulated monocytes developing into macrophages to the ALI cultures. Our model systems recapitulate key features of the human respiratory epithelium, in which we we have investigated how host–pathogen interactions shape antibiotic responses in *Pseudomonas aeruginosa*. In parallel, we examined the functional consequences of clinically relevant resistance mutations on bacterial behavior in this host-relevant environment.

Results: In the ALI model systems, we have shown how mutations in a multidrug efflux pump regulator facilitate bacterial protection from antibiotics through enhanced tissue invasion, and how loss-of-function mutations in the carbapenem-entry porin OprD remodeled outer-membrane architecture and altered bacterial engagement with the epithelial surface, significantly enhancing colonization capacity.

Conclusions: Altogether, these results underscore that antibiotic resistance and host–pathogen interactions are tightly interconnected processes: resistance mutations can reshape infection dynamics, while host-driven adaptation can modulate resistance phenotypes. Therefore, it is of critical importance to understand host-pathogen interactions in the CF airways, and our research highlights the need to consider host-pathogen interactions when evaluating resistance during infection. The proposed ALI model systems are relevant infection models to accurately assess drug efficacy and resistance development.

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S 6.4 Pro-repair strategies targeting CFTR and K+ channels and counteracting bacterial virulence to restore airway epithelial integrity in cystic fibrosis

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Background: Pulmonary disease remains the major cause of morbidity and mortality in cystic fibrosis (CF) and the recovery of lung function depends on efficient repair of airway damage, particularly in individuals with advanced disease. Beyond its essential role in regulating ion and fluid transport, necessary for mucociliary clearance, CFTR also contributes directly to epithelial repair. Therefore, both the CFTR defect and the presence of chronic infection impair the capacity of the CF airway epithelium to repair effectively after injury. Pro-repair therapeutic strategies are thus needed to restore lung integrity.

Previous work from our group showed that functional rescue of CFTR using modulators, as well as complementary approaches targeting K+ channels or reducing bacterial virulence, can promote epithelial repair.

Objective: We aimed to dissect how CFTR regulates epithelial repair and to determine how CFTR modulators improve early and late repair processes. We also sought to evaluate combined approaches involving CFTR modulation, K+ channel activation, and strategies designed to attenuate bacterial virulence.

Methods and results: Early and late repair processes (including cell migration and proliferation of progenitor cells, repair/regeneration of an intact, differentiated, ciliated epithelium) were assessed using primary airway epithelial cell cultures derived from people with CF (pwCF) carrying a range of CFTR mutations. Our results confirm that epithelial repair is impaired in CF. Functional rescue of CFTR with modulators (Lumacaftor/ Ivacaftor or Elexacaftor/Tezacaftor/Ivacaftor) improved guided cell migration at the wound-edge, associated with activation of key proteins regulating the cell migration process. CFTR rescue also accelerated wound closure and promoted regeneration of a tight and ciliated epithelium after chronic treatments.

Combined approaches decreasing the virulence of P. aeruginosa and S. aureus preserved epithelial repair under infectious conditions, supporting the relevance of targeting pathogen-driven impairment of healing. Importantly, activation of K+ channels exerted significant pro-repair effects in CF cultures, with mutations non-responding to CFTR modulators, demonstrating CFTR-independent pathways can restore epithelial capacity.

Conclusions: Together, these findings demonstrate that CFTR function is central regulator of airway epithelial repair and that functional rescue of CFTR substantially improves both early and late stages of tissue repair. K+ channel activation and attenuation of bacterial virulence further enhance repair, including in settings where CFTR modulators alone are not efficient. These results support the development of combination therapeutic strategies, adapted to CFTR mutations, aimed at restoring robust epithelial repair in CF, with potential benefits for patients with advanced pulmonary disease or limited responsiveness to current CFTR modulators.

Acknowledgements: CIHR, AIRS Network.

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S 6.5 Advanced endometrial organoid models to unravel endometrium-linked fertility problems in cystic fibrosis

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Background: Women with CF (wwCF) experience notable fertility challenges, showing higher rates of infertility compared to healthy women (35% vs. 5-15%). Since the introduction of highly effective CFTR modulator therapy, pregnancy rates have increased in wwCF. However, underlying causes of fertility problems in wwCF and effects of CFTR modulator therapy on the female reproductive system remain poorly understood. In particular, the pathobiology of the endometrium, the uterus' inner lining essential for embryo implantation toward successful pregnancy, remains largely unexplored in CF. Recently, our lab developed organoids from the endometrium of wwCF (CFEO), which were found to display CF characteristics, interesting transcriptomic differences with healthy endometrium-derived organoids (HEO), and rescue responses to CFTR modulators.

Aims and Methods: In the present study, we employ CFEO to establish advanced models to explore potential aberrations in reproductive processes including embryo implantation and sperm capacitation in CF. The models are compared to the healthy condition, and the impact of CFTR modulators is explored. The early stages of implantation are assessed using our previously developed in vitro embryo implantation model, consisting of stem-cell derived blastocyst models (blastoids) which are added on top of EO-derived 'open-faced endometrial layers' (OFEL) with which the blastoids physiologically interact. In addition, we are establishing an endometrium-sperm transwell co-culture system to analyze the impact of endometrial CFTR dysfunction (resulting in altered pH and ion transport) on sperm motility and capacitation, a process needed to render sperm capable of egg fertilization which in vivo occurs in the uterus.

Results: The first step of embryo-endometrium interaction, i.e. attachment of the blastoid to the OFEL, was found to be significantly reduced in CF versus healthy (fertile) condition. Our current analyses explore whether specific molecular pathways previously identified in CFEO contribute to this impaired implantation and whether CFTR modulators can restore this deficient interaction. Interestingly, preliminary findings indicate an increase in blastoid attachment to CF OFEL when treated with Elexacaftor+Tezacator+Ivacaftor (ETI).

To investigate the impact of CFTR dysfunction in the uterine environment on sperm cells, we are currently comparing the pH in CFEO vs. HEO using a pH-sensitive probe and FLIM microscopy. To assess ion transport in CF vs. healthy OFEL, we apply the Ussing chamber assay. Our first data reveal elevated ENaC activity and lower CFTR activity in CF, both of which appear normalized by ETI, thereby validating the applicability of the OFEL model to examine ion transport across the endometrium in CF.

Conclusions: By leveraging organoid-derived models, our work will advance mechanistic understanding of CF-associated reduced fertility. Given that CFTR modulators extend life expectancy and increase reproductive opportunities in people with CF, deeper insights are considered very timely and clinically relevant, informing future strategies to support family planning of wwCF

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S 6.6 Organotypic in vitro model of the human lung using hiPSCs to study epithelial-macrophage interactions and bacterial infections of the upper respiratory tract in cystic fibrosis

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Model systems for the respiratory tract are essential for reliable cystic fibrosis (CF) research and so far rely on animal studies, primary or immortalized cells. However, these approaches fail to capture the complexity of lung physiology while maintaining scalability and relevance to the human disease phenotype. To address these limitations, our group developed a CF lung model using induced pluripotent stem cell (iPSC) technology. Despite the advantages hiPSCs provide, a lack of complex in vitro models that combine respiratory epithelial cells with components of the immune system, particularly macrophages, remains. To bridge this gap, this study seeks to build and characterize an organotypic in vitro model for the lung (AIRMAC) using hiPSC-derived airway epithelial cells (iAECs) and thereof derived Air-Liquid-Interface (iALI) cultures in combination with hiPSC-derived macrophages (iMacs), both carrying the CF genotype and its isogenic control to investigate intrinsic and extrinsic contributions of the CFTR mutation on macrophage and airway epithelial functionality. AIRMAC combines two highly differentiated systems, closely resembling their primary cell counterparts at molecular and functional levels while enabling long-term survival of both cell types for at least 25 days. Within AIRMAC, macrophages exhibit lateral mobility and cluster formation on the iAEC layer, behaviors analogous to in vivo macrophage activity. Current efforts focus on assessing the plasticity and functionality of iMacs in the AIRMAC system including transcriptional and secretome analyses as well as studies on the pathogen and mucus uptake in a time dependent manner. Our lung model is providing a scalable platform for investigating bacterial infections of CF patients in a human-relevant context.

14 March - 11:00 - 12:45

Symposium 7
Systems Biology & Multi-omics and CFTR structure

S 7.1 Beyond the ion channel: systems-level insights into CFTR and epithelial transformation

Cláudia Rodrigues, Raquel Torres, Ines Pankonien, Margarida D. Amaral

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Cystic fibrosis (CF) has long been considered a paradigmatic monogenic channelopathy caused by mutations in the gene encoding CFTR, an apical epithelial anion channel. However, accumulating evidence indicates that CFTR dysfunction extends far beyond impaired ion transport, reshaping epithelial biology at a systems level. High-throughput and multi-omics approaches have begun to reveal how CFTR integrates into complex molecular networks governing epithelial differentiation, polarity, regeneration, and disease progression. Integrating these large-scale datasets into coherent disease maps is essential to uncover mechanistic links and identify novel, unbiased therapeutic targets.

Along these lines, beyond its canonical channel function, CFTR plays a critical role in maintaining epithelial integrity, preventing epithelial-to-mesenchymal transition (EMT), and suppressing tumorigenesis. CF tissues exhibit developmental abnormalities, defective wound repair, and increased cancer susceptibility, supporting the classification of CFTR as a tumour suppressor. Our recent studies demonstrate that CF airway epithelial cells undergo a partial, active EMT that is directly driven by CFTR dysfunction and occurs independently of secondary factors such as infection or inflammation. This EMT state is characterized by impaired epithelial differentiation, reduced junctional integrity, altered cytoskeletal organization, and dysregulated proliferation.

Our recent mechanistic studies showed that both the absence of CFTR from the plasma membrane (PM) and the loss of its ion transport activity contribute to EMT. However, the most severe phenotype is observed when CFTR fails to localize to the membrane (Phe508del-CFTR). Indeed, PM-localized but nonfunctional CFTR (Gly551Asp-CFTR) partially preserves epithelial features, indicating that PM CFTR exerts structural and signalling roles in addition to ion transport. We previously also showed that CF-EMT occurs with the activation of key EMT-related transcription factors (EMT-TFs), including YAP1, TEAD4, and TWIST1. Our emerging interactome and proteomic analyses have further linked CFTR to EMT through protein networks involving mediators such as fibronectin 1 and keratin-18. Of relevance, CFTR modulators only partly revert the EMT state, thus suggesting that additional therapeutic strategies or targets are likely required.

Together, these findings reposition CFTR as a central organizer of epithelial identity whose dysfunction triggers widespread transcriptional and proteomic reprogramming. Understanding CF as a disorder of epithelial transformation rather than solely an ion transport defect opens new avenues for therapeutic strategies that combine CFTR modulation with targeting of EMT-related pathways to restore full epithelial integrity.

S 7.2 Molecular mechanisms of CFTR channel regulation by protein kinase A

Làszlo Csanady

Semmelweis University

The CFTR anion channel comprises two transmembrane domains (TMD1, TMD2), two cytosolic nucleotide binding domains (NBD1, NBD2), and a regulatory (R) domain. The pore, formed by the TMDs, opens upon formation of a tight NBD1/NBD2 heterodimer following ATP binding, and closes upon disruption of the NBDs are continuously exposed to saturating ATP, and channel activity is regulated by its interaction with the catalytic subunit of protein kinase A (PKA). PKA activates CFTR channels though two additive mechanisms: noncatalytically, through direct binding, and catalytically, by phosphorylating multiple target serines in CFTR's regulatory (R) domain. The unphosphorylated R domain is wedged between the two NBDs, preventing their dimerization. PKA binding and R domain phosphorylation additively promote release of the R domain from its inhibitory position. Recent functional and structural studies have identified important molecular determinants of CTFR channel activation by PKA, and dissected the kinetics and relative contributions of noncatalytic channel activation for wild-type (WT) CFTR. New functional data will be presented, addressing how those two processes are affected by the two common cystic fibrosis (CF) associated CFTR mutations F508del and G551D, by the ATP analog N^6 (2 Phenylethyl)-ATP (P-ATP), and by ivacaftor and elexacaftor, the two components of the Kaftrio formulation that possess potentiator activity. Mechanistic implications will be discussed.

S 7.3 Omics data and systems biology approaches in the context of CF

Véronique Stoven[1,2,3], Mairead Kelly-Aubert[4,5,6], Victor Laigle[1,2,3], Matthieu Najm[1,2,3], Loredana Martignetti[2,3], Laurence Calzone[2,3] , Isabelle Sermet-Gaudelus[4,5,6]

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Cystic Fibrosis (CF) is caused by mutations in the cftr gene, but its overall physio-pathology cannot be solely explained by the loss of the CFTR chloride channel function. Indeed, CFTR belongs to a yet not fully deciphered network of proteins participating in various signalling pathways. We make the hypothesis that absence of a functional CFTR protein perturbs its direct partners, which propagate further molecular deregulation in the network, resulting in a panel of deleterious CF cellular phenotypes.

Based on omic data from F508del CF airway epithelial cell and their controls, we identified signalling pathways up- or down-regulated in CF cells. Using systems biology approaches and biological pathway databases, we connected these pathways into a network. Presence in this network of several proteins known to interact with CFTR but not with F508del-CFTR is consistent with our hypothesis. The network comprises proteins, and vertices corresponding to functional relations between them (activation, inhibition, phosphorylation of a protein by a kinase etc…).

Topological analysis of the network highlighted three types remarkable proteins: (1) sources, i.e. direct protein interactors of CFTR, that initiate deregulations; (2) hub proteins towards which many vertices converge and from which many vertices leave, and that propagate deregulations in the network; (3) outputs, i.e. proteins (mainly transcription factors) from which no vertices leave and that modulate downstream deleterious cellular phenotypes including inflammation, perturbed innate immunity, or deregulation of actine cytoskeleton dynamics.

Analysis of the network allowed identification of new candidate therapeutic targets among source and hub proteins for which known drugs are available. These candidate targets are currently evaluated based on several experimental approaches on CF cellular models.

P 3

S 7.4 Exploring the cellular pathways to promote rescue of mutant CFTR protein in cystic fibrosis

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CFTR stabilization at the plasma membrane (PM) represents a promising yet underexplored strategy to improve the rescue of mutant CFTR proteins implicated in cystic fibrosis (CF). At the PM, CFTR associates with numerous protein partners, which coordinate cAMP-dependent signaling crucial for CFTR regulation. Proper cytoskeletal integrity provides the necessary scaffolding to stabilize CFTR and recruit key regulatory proteins such as the cAMP sensors PKA and EPAC1 [1]. Among the cytoskeletal components in close proximity to CFTR, we identified the capping protein CAPZA2 and the inverted formin INF2 – with the first being a positive regulator and the second a negative regulator of CFTR stability at the PM [2]. Our aim here is to elucidate how the crosstalk between the cytoskeleton and cAMP signaling regulates CFTR trafficking by investigating the role of EPAC1, CAPZA2, and INF2 in CFTR trafficking and localization at the PM. Using primary nasal epithelial cell cultures from people with (pwCF), CF bronchial epithelial cells expressing wild-type or mutant CFTR, assays including Western blotting, co-immunoprecipitation, and cell surface biotinylation were performed. Live cell imaging assessed cAMP pools, and proximity labeling techniques identified INF2 interactors. Results showed that knockdown of INF2 and CAPZA2 had a particular impact in membrane-associated cAMP pools in both wild-type and F508del CFBE cells (increase for INF2 and decrease for CAPZA2). Activation of EPAC1 increased PM wt-CFTR association with NHERF1, whereas the opposite effect was observed for F508del-CFTR. INF2 lower molecular weight isoforms were detected in native cells, and ongoing interactomics studies by proximity labeling suggests an enrichment of cytoskeleton proteins among ER INF2 interactors in CFBE cells. Furthermore, a bioinformatic analysis using an MS/MS-based method ranked proteins relevant to CFTR PM stabilization and identified novel potential regulators. Together, these findings reveal the complex regulation of CFTR by EPAC1, INF2, and CAPZA2, underscoring the critical need to understand their roles in cytoskeleton-cAMP signaling crosstalk to modulate CFTR function and potentially improve CF handling.

Acknowledgements: Work supported by FFC Ricerca grant FFC#2/2023 and by FCT, Portugal through UID/04046/2025 center grant (to BioISI) and PhD fellowship 2021.06174.BD to JFF. Mass spectrometry data for INF2 interactome were generated by the Mass Spectrometry Unit (UniMS), ITQB/iBET, Oeiras, Portugal.

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[2] Santos JD et al (2020) Biochem J 477, 2561–80. https://doi.org/10.1042/BCJ20200287.

P 9

S 7.5 ATP-dependent modulation of the NBD1 conformational equilibrium from the CFTR channel

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Cystic fibrosis transmembrane conductance regulator (CFTR) is a channel that conducts chloride and bicarbonate ions and regulates fluid transport across cell membrane in epithelial tissues. Its gating is governed by phosphorylation and ATP binding and hydrolysis at its cytosolic nucleotide-binding domains (NBDs). CFTR possesses asymmetric ATPase sites: NBD2 is the consensus site that binds and hydrolyzes ATP, while NBD1 hosts a degenerate ATP site which lost its ability to hydrolyze ATP due to evolutionary mutations. CFTR is a unique among ABC-transporters in containing a disordered regulatory insertion (RI) – a 32-amino acid segment located in NBD1 near the ATP-binding site. The functional role of the RI remains incompletely understood. Our current work aims to elucidate the role of ATP binding in NBD1.

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 interpreted 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.

Differential scanning fluorimetry (DSF) confirmed that the W401A mutant exhibits decreased thermal stability compared 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 experiences loss of structure and ATP is entirely absent from its expected binding site of the domain. The potential effects of the stabilizing nanobody on the conformational equilibrium in the domain were excluded using the smFRET approach.

Using the hydrogen-deuterium exchange coupled to mass spectrometry (HDX-MS) approach, we identified peptides in NBD1 that exhibit reduced deuterium exchange in the ATP presence. These peptides are in close proximity within the tertiary structure and potentially play a role in NBD1 stabilization through physical contact between the α- and β-subdomains.

The triphosphate moiety and the adenine base of ATP both contribute to NBD1 stabilization, where the adenine base alone plays a role as a modulator of the conformational equilibrium within the domain.

To further elucidate the possible biological meaning of the conformational equilibrium, a single-channel activity assay on the full-length W401A mutant channel would be needed.

P 100

S 7.6 Systemic effects of cystic fibrosis transmembrane conductance regulator (CFTR) modulators on the plasma and serum proteome

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Cystic fibrosis (CF) is caused by a dysfunction in the cystic fibrosis transmembrane conductance regulator (CFTR) and affects multiple organs by obstructing mucus and altering secretion. While the combination drug elexacaftor/tezacaftor/ivacaftor (ELX/TEZ/IVA, ETI) has markedly improved clinical symptoms, its broader molecular and systemic effects remain to be fully elucidated.

Using mass spectrometry-based proteomics, we compared the plasma and serum proteomes of people with cystic fibrosis (pwCF) who were treated with the earlier, less effective lumacaftor/ivacaftor (LUM/IVA) combination with those who received the more potent ELX/TEZ/IVA therapy. Our analysis revealed specific and common pharmacodynamic signatures associated with inflammation and metabolic processes under each treatment regimen. Notably, the ELX/TEZ/IVA therapy produced more consistent alterations in pwCF that resembled profiles observed in healthy individuals.

Furthermore, by comparing the sputum and serum proteomes of patients with cystic fibrosis (pwCF) treated with ELX/TEZ/IVA, we identified counter-directional changes in pulmonary surfactant-associated protein B (SFTPB), a potential biomarker of lung permeability. These changes correlated with improvements in lung function and could be validated in an independent cohort.

This study provides a comprehensive resource that enhances our understanding of CFTR modulator-driven proteome alterations. It offers insights into systemic and local protein regulation in cystic fibrosis (CF). Our findings suggest that ELX/TEZ/IVA promotes broader systemic health improvements and provide critical information that could inform future CF therapies.

14 March - 14:15 - 16:00

Symposium 8
Infection and Immunology in the CFTR modulator era

S 8.1 Adaptation of Staphylococcus aureus during chronic infection of the lung

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Chronic lung infection with *Staphylococcus aureus* is common in cystic fibrosis (CF) and can persist for years despite antimicrobial therapy. Bacterial persistence is accompanied by reproducible shifts in virulence regulation, metabolism and host interaction, yet the airway-derived selective pressures and their impact on inflammation remain incompletely defined. Here, we integrate recent genomic, transcriptomic and functional evidence to define mechanisms that promote chronic, persistent colonization by S. *aureus* in the CF airway.
Comparative genomics of sequential isogenic isolates recovered 2–9 years apart from chronically infected CF children demonstrates within-host evolution with convergent mutations affecting carbohydrate metabolism, cell-wall remodelling, genetic information processing and adhesion.
A central convergent route is loss of quorum sensing activity through dysfunction of the accessory gene regulator (agr) system. In the CF airway, highly sialylated mucins coexist with sialidase-producing members of the microbiota, generating free sialic acid as an exploitable nutrient. Agr-defective variants display increased sialic acid catabolism, gain a competitive growth advantage in vitro and in vivo, and are selectively enriched when free sialic acid is available. Beyond serving as a carbon source, exposure to free sialic acid drives transcriptional reprogramming consistent with a chronic infection program, linking nutrient availability to regulatory rewiring.
Importantly, lung-adapted late isolates display reduced cytotoxicity compared with early isolates yet trigger stronger epithelial pro-inflammatory cytokine secretion. This response is driven by increased expression of staphylococcal protein A (Spa), which is derepressed upon agr dysfunction, and requires TNF-α receptor 1 signalling.
Together, these findings indicate that chronic airway colonization drives convergent evolutionary strategies coupling metabolic opportunism with quorum-sensing rewiring, shifting from acute toxicity toward persistence and sustained inflammation.

S 8.2 Is inflammation still a target in CF?

Robert Gray

School of Infection and Immunity, University of Glasgow, Scotland, UK

Cystic fibrosis lung disease is characterized by repeated cycles of infection and inflammation that cause lung damage. CF modulator drugs have improved the lives of many people living with CF, but airway infection and inflammation persist, even in those eligible for modulator therapy. Despite several decades of work, we don't understand inflammation in CF, how best to measure it, and perhaps most importantly, how to treat it. Advances in analytical biology allow us, for the first time to visualize inflammation at a tissue level and probe the potential interaction of immune cells and epithelial cells in the inflamed lung.

This talk will review the present evidence of how inflammation has changed with CFTR modulators but also which inflammatory proteins may remain as targets in the post-CFTR modulator era. I will present new data demonstrating how spatial biology techniques can be used to visualize cell populations in the CF lung and how they interact at a transcriptional level. Furthermore, I will present our latest data on how the major CF biomarker calprotectin is a potential target for precision anti-inflammatory drug development.

This presentation will share unpublished data from my lab that builds on our previous CF inflammation publications and suggest future avenues for inflammation research and drug development with an aim to improving lung health for people with CF, including those unable to take CFTR modulators.

S 8.3 Reimagining Infection Management in CF with Phage Therapy: From Biology to Clinical Application

Katrine Whiteson

University of California, Irvine

Antibiotic resistance continues to jeopardize infection control, especially for people with cystic fibrosis (pwCF) and others with chronic airway disease. Bacteriophage (phage) therapy is gaining traction as a promising alternative, particularly for drug-resistant pathogens such as *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia*, and *Staphylococcus aureus*, which commonly infect pwCF. Case studies across infection types demonstrate clinical improvement in 60-80% of patients, even in refractory cases.

Our team at UC Irvine has built a robust phage discovery and production pipeline, collecting and characterizing phages from Southern California wastewater for over a decade. We have discovered and sequenced dozens of phages, profiled host ranges, and matched phages to infecting strains. In 2024, we successfully treated a chronic *S. aureus* sinus infection using a newly isolated phage. We will describe this experience and review CF-relevant phage therapy cases and clinical trials.

Furthermore, we will present research efforts to further expand phage utility. One persistent challenge is the narrow host range of individual phages. To overcome this, we are applying experimental evolution approaches to adapt phages in the laboratory, enabling them to infect previously resistant clinical isolates. Evolved phages often show broader host range and enhanced efficacy against patient-derived strains. In parallel, we are exploring molecular adjuvants that enhance phage infectivity. Medium-chain fatty acids (MCFAs), naturally occuring antimicrobial molecules found in diet and surfactants, have demonstrated promising synergy with phages and antibiotics across a wide array of CF-relevant pathogens. Across a collection of clinical isolates including 18 *S. maltophilia*, 14 *P. aeruginosa*, 18 *S. aureus* and 91 *Enterococcus* strains, MCFA and phage combinations demonstrated board-spectrum inhibition.

Ongoing work includes studies of phage-adjuvant-antibiotic interactions, experimental evolution to expand host range via phage training, screening of additional synergistic compounds, and broader efforts to advance both the scientific foundations and clinical readiness of phage therapy for CF and beyond.

P 96

S 8.4 The PD-1 / SHP2 immune checkpoint controls bacterial killing by human CF airway neutrophils

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Objectives: People with CF (pwCF) suffer from chronic airway infections in spite of the massive presence of neutrophil phagocytes in the lung lumen. This paradox is explained by our discovery of an acquired, active tolerance of bacteria by neutrophils recruited to the CF airway milieu (Margaroli, Cell Rep Med 2021). Here, we tested whether this tolerance was controlled, at least in part, by the PD-1 / SHP 2 axis, a well-known immune checkpoint signaling pathway in cancer and autoimmune diseases.

Methods: PD-1 expression was assessed in primary blood and sputum neutrophils from pwCF by flow cytometry. In addition, CF airway supernatant (CF ASN, obtained from sputum, per Dobosh, STAR Prot 2021) was used to transmigrate and condition human blood neutrophils to adopt a bacteria tolerant phenotype. Next, CFASN-conditioned neutrophils were assessed for activation of the SHP2 phosphatase downstream on PD-1. Rescue of their bactericidal activity against P. aeruginosa (CF pathogen) was attempted by treatment with an anti-PD1 antibody combined with a SHP2 inhibitor.

Results: PD-1 and its major ligand PD-L1 are highly upregulated on sputum compared to blood neutrophils collected from pwCF. At the transcriptional levels, human blood neutrophils conditioned into CF airway neutrophils in our biomimetic model show a steady increase in SHP2 expression over the course of 2 to 14 hours of transmigration and exposure to CFASN. Remarkably, blockade of the PD-1 / SHP2 axis in these CF airway-conditioned neutrophils restores killing of P. aeruginosa to the level observed in control airway neutrophils.

Conclusion: The PD-1 / SHP2 axis is involved in the inhibition of bacterial killing and resulting bacterial tolerance by neutrophils recruited to the CF airway lumen. PDL-1 on neutrophils is the likely ligand for activation of this checkpoint, as we previously showed for PD-1 ligation on macrophages in infants with CF (Margaroli, J Cyst Fibros 2022). Our data support further exploration of PD-1 pathway inhibitors, repurposed from other domains of medicine, as a mutation-agnostic immunotherapy in CF.

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+ Winship Flow Cytometry Core.

P 41

S 8.5 Long-standing adaptation and regulatory plasticity in Pseudomonas aeruginosa during CFTR restoration

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Elexacaftor/tezacaftor/ivacaftor (ETI), a highly effective CFTR modulator combination, is transforming cystic fibrosis (CF) care, driving profound improvements in lung function, exacerbation burden, and quality of life. Yet, despite these gains, chronic Pseudomonas aeruginosa infection often persists in people with CF (pwCF) beginning ETI, revealing resilience to the improved airway environment. Additionally, therapeutic benefit varies widely among individuals, prompting renewed investigation into microbial contributions to treatment response.

To investigate pathogen adaptation under CFTR restoration, we implemented a longitudinal framework integrating phenotypic assays, whole genome (WGS) and transcriptome (RNAseq) sequencing, and clinical outcome analysis. Fourteen chronically infected people with CF initiating ETI contributed sputum samples before and during therapy. A total of 54 P. aeruginosa isolates were collected, and 35 isolates from 11 participants underwent short- and long-read WGS to construct personalized reference genomes and track within-host evolution over time. Phenotypic traits associated with chronic infection, including motility, biofilm formation, pyocyanin and protease production, mucoidy, growth rate, and antibiotic susceptibility, were systematically evaluated. Six isolates were additionally exposed in vitro to ETI to identify transcriptional responses directly induced by modulators, assessed by RNAseq. Clinical outcomes were analyzed using 12-month windows: the year before ETI initiation and the year preceding the final post-ETI sampling for each participant. Relationships between pathogen and pwCF features were explored using Factorial Analysis of Mixed Data.

Classical chronic phenotypes persisted across isolates, including mucoidy, multidrug resistance, reduced motility, and lasR-associated traits. Genomic diversification continued, but with minimal overlap of mutated genes across individuals, consistent with individualized evolutionary trajectories.

Transcriptomic profiling revealed strain-specific yet pathway-convergent changes involving metabolism, virulence, stress responses, and β-lactam resistance, suggesting shared selective pressures despite divergent genetic routes. Several of these transcriptional signatures also appeared after in vitro exposure to ETI, supporting the possibility that CFTR modulators exert direct effects on bacterial regulatory pathways. Phenotypic assays did not reveal corresponding changes under the tested conditions, suggesting that early and reversible regulatory adjustments may require more prolonged exposure to translate into detectable traits.

Clinically, participants experienced significant improvement, including a median 15.5% increase in FEV percent predicted and a reduction in exacerbations, hospitalizations, and intravenous antibiotic use. Individuals with the greatest benefit tended to harbor isolates with loss or absence of mucoidy and the LasR-associated phenotype, persistent or emerging hypermutability, and reduced pyocyanin production, suggesting that pathogen features may relate to treatment efficacy. These findings show that while ETI markedly improves the airway environment and clinical outcomes, P. aeruginosa persists through deeply rooted adaptations and responds primarily through flexible regulatory rewiring. Monitoring pathogen evolution during ETI therapy may support precision management of chronic infection in the evolving CF landscape.

This study was supported by the Italian CF Research Foundation (FFC#16/2021).

P 45

S 8.6 modulates airway inflammation and reduces Pseudomonas aeruginosa burden in pediatric cystic fibrosis

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Background: In cystic fibrosis (CF), inflammation drives disease progression. This heightened inflammatory state results from several converging mechanisms, including direct pro-inflammatory effects of CFTR mutations, epithelial hypoxia arising from mucus stasis, and pathogen-induced inflammation. Pseudomonas aeruginosa (Pa), the most common bacterial pathogen in CF, further amplifies airway damage by triggering neutrophil recruitment and protease release, notably neutrophil elastase (NE). While the anti-inflammatory properties of triple combination therapy (ETI) are increasingly recognised, its impact on host immune defences and bacterial burden remains insufficiently characterised.

Aims: This study aimed to evaluate how treatment controls Pa burden and reshapes innate immune responses in children with CF.

Methods: Induced sputum samples were collected within the multicenter observational study MODUL-CF (NCT04301856). Longitudinal sputum cultures were analysed at baseline and after 12 months of ETI therapy allowing classification of patients as chronic, intermittent, or negative. To assess the impact of ETI on bacterial burden and host defence, sputum concentrations of antimicrobial peptides (Cathelicidin, SLPI and β-defensins) were measured before and after therapy alongside NE, calprotectin, IL-1β, IL-6, IL-8 and TNF-α, and were compared to 13 healthy controls. To complement these in vivo experiments, primary nasal epithelial cells from healthy donors, Phe508del individuals, and patients carrying a premature termination codon (PTC) were cultured at the air-liquid interface (ALI), treated with ETI or vehicle (DMSO) and infected with Pa. Bacterial growth was monitored across genotypes with and without treatment.

Experiments were repeated in the presence of a neutralizing anti-SLPI antibody to assess the contribution of this major antimicrobial peptide, and supernatants were collected to quantify inflammatory markers.

Results: Notably, after 12 months of ETI therapy, and in the absence of concurrent antibiotic treatment, a subset of patients cleared Pa from their airways. In a separate cohort, sputum concentrations of the antimicrobial peptide SLPI increased significantly after treatment ($p<0.01$), accompanied by reductions in pro-inflammatory markers (NE, calprotectin, IL-1β, IL-6, IL-8, and TNF-α) although levels remained above those observed in healthy controls. Importantly, the pattern observed in ECBC data was recapitulated ex vivo in Pa-infected primary epithelial cells. Incubation with ETI reduced bacterial growth in cells from CF patients ($p<0.05$), but not in cells from healthy donors ($p=0.73$) or individuals carrying a PTC ($p=0.55$). Notably, the observed reduction was not due to a direct antibiotic effect, suggesting that ETI induces host-mediated mechanisms limiting Pa growth. Neutralization of SLPI with a specific antibody reduced the ETI-mediated inhibition of bacterial growth, suggesting a key role for SLPI in controlling Pa under therapy.

Conclusion: ETI not only exerts potent anti-inflammatory effects but also enhances host antimicrobial defences, suggesting a dual role in limiting infection and shaping airway innate immunity in children with CF.

14 March - 16:30 - 17:45

Closing Keynote lecture

Ethics in research: feedback from the bench

Bruno Clement

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Ethics in research is not just an intangible requirement derived from philosophy, history, and international recommendations, or imposed by law and ad hoc committees. It is also a daily responsibility that comes into play at every stage of research, from project design to experimentation (animal and human), then publication in specialized journals or communication to the general public. Research does not always have immediate applications or direct benefits for patients. It is indeed a very long process involving many people, scientific disciplines, and areas of expertise. At the end of this process, each advance in knowledge can have a greater or lesser impact on the entire scientific and medical community, and then on all citizens. In addition, researchers, whether involved in basic or clinical research, must be aware that their work and discoveries may be applied in ways they cannot fully predict. Furthermore, the production of indisputable scientific facts is an essential component of Democracy in the 21st century. Researchers therefore have an ethical responsibility in their daily work.

P 1

ATP-independent activity of elexacaftor-tezacaftor-ivacaftor-rescued F508del-CFTR

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The triple-combination therapy elexacaftor-tezacaftor-ivacaftor (ETI) has transformed the treatment of people with Cystic Fibrosis (CF) by rescuing the folding and function of faulty Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) Cl^- channels. Gating of wild-type (WT) CFTR is tightly coupled to cycles of ATP binding and hydrolysis following phosphorylation of the regulatory domain. However, gating of some CFTR variants (e.g. G551D) is ATP-independent (1).
We investigated the PKA- and ATP-dependence of single ETI-rescued F508del-CFTR channels using the patch-clamp technique. Inside-out membrane patches were excised from Baby Hamster Kidney (BHK) cells stably expressing F508del-CFTR. Prior to study, BHK cells were incubated for 24 h at 37 °C with E (2 µM), T (3 µM) and I (1 µM). Membrane patches were exposed to a large [Cl^-] gradient in the continuous presence of acutely added E (2 µM) and I (100 nM) and voltage clamped at -50 mV. Channels were activated at 25 °C before temperature was increased to 37 °C. Data are means ± SD.
In the presence of both ATP (1 mM) and PKA (75 nM), the open probability (P_O) of ETI-rescued F508del-CFTR was WT-like (0.6 ± 0.2; n = 8; 37 °C). By contrast, a vanishingly low P_O (0.01 ± 0.01; n = 6; 25 °C) was observed in the absence of PKA, but presence of ATP, which was restored to WT-like levels (P_O = 0.4 ± 0.2; n = 6; 25 °C) following acute addition of PKA (75 nM). However, excising membrane patches into an intracellular solution containing no acutely added ATP, in the presence of PKA, revealed a modest P_O (0.1 ± 0.05; n = 6; 37 °C), which increased with [ATP]. Of note, ATP-independent channel activity was mediated predominantly by subconductance state (SC-S) openings. SC-S behaviour was defined by: i) fast transitions between at least three subconductance levels; ii) [ATP]- and modulator-dependence; iii) clear changes in behaviour over time. Strikingly, the gating mode of F508del-CFTR defined by marked SC-S activity was also observed following the onset of channel deactivation, during prolonged recordings, in the presence of E, I and ATP. This suggests that ATP not only shifts ETI-rescued F508del-CFTR's equilibrium to favour opening over closing, but also stabilises a gating mode characterised by full conductance rather than subconductance openings.
These findings reveal an alternate mode of CFTR behaviour, which re-frames channel gating models. Further studies will be required to illuminate the precise contributions of PKA, elexacaftor and ivacaftor to this behaviour, its relevance to people with CF and F508del-CFTR receiving ETI, and transport cycles of ABC family transporters.
Supported by the CF Trust.

References: Bompadre et al. *J Gen Physiol.* 2007;129(4):285-98.

P 2

Impact of elexacaftor-tezacaftor-ivacaftor on pig F508del-CFTR, a Cl- channel with greater residual activity than human F508del-CFTR

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Cystic fibrosis transmembrane conductance regulator (CFTR) function and pharmacology differ across species (1–2). To inform studies of CFTR modulators using pig models of cystic fibrosis (CF), here we investigated pig F508del-CFTR and its response to elexacaftor-tezacaftor-ivacaftor. We transiently expressed CFTR variants in CHO cells and studied their single-channel behaviour in excised inside-out membrane patches using a large Cl^- concentration gradient ($[Cl^-]_{int}$, 147 mM; $[Cl^-]_{ext}$, 10 mM) with voltage clamped at –50 mV and ATP (1 mM) and PKA (75 nM) added to the intracellular solution; temperature was 37 °C. When compared with human wild-type CFTR, pig wild-type CFTR formed Cl^- channels with greater activity characterised by prolonged bursts of channel openings of larger amplitude (n = 13–15). After treating F508del-CFTR-expressing cells with elexacaftor (2 µM), tezacaftor (3 µM) and ivacaftor (1 µM) for 24 h at 37 °C, human F508del-CFTR Cl^- channels had a bursting pattern of channel gating with an open probability (P_o) one third that of human wild-type CFTR and a current amplitude similar to that of human wild-type CFTR (n = 7–13). By contrast, elexacaftor-tezacaftor-ivacaftor-rescued pig F508del-CFTR Cl^- channels had a bursting pattern of channel gating characterised by infrequent prolonged channel openings with a P_o half that of pig wild-type CFTR and a current amplitude larger than that of pig wild-type CFTR (n = 9). For both human and pig F508del-CFTR, following chronic treatment with elexacaftor-tezacaftor-ivacaftor, channel activity was stabilised in excised inside-out membrane patches at 37 °C. To validate these findings at the population level, we transiently expressed pig wild-type and F508del-CFTR in Fischer rat thyroid (FRT) cells and measured CFTR-mediated Cl^- transport with the Ussing chamber technique. Three days after seeding transfected FRT cells on filter supports, when R_t >2,000 $\Omega \cdot cm^2$, we treated FRT epithelia with either DMSO (0.3% $v \cdot v^{-1}$) or elexacaftor (2 µM), tezacaftor (3 µM) and ivacaftor (1 µM) for 24 h at 37 °C. When compared with DMSO-treated F508del-CFTR-expressing FRT epithelia, elexacaftor-tezacaftor-ivacaftor enhanced forskolin-stimulated Cl^- current by 10-fold (n = 4), producing responses comparable to those of FRT epithelia expressing pig wild-type CFTR (n = 3). Consistent with species-dependent differences in inhibitor sensitivity (3), $CFTR_{inh}$-172-inhibited CFTR-mediated Cl^- current was smaller in FRT epithelia expressing pig than human CFTR. In summary, the F508del variant has species-specific effects on the single-channel behaviour of CFTR. Elexacaftor-tezacaftor-ivacaftor enhances current amplitude in pig F508del-CFTR but improves gating only in human F508del-CFTR. These findings highlight important cross-species differences in CFTR structure-function relationships relevant to CF animal models.

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P 3

Exploring the cellular pathways to promote rescue of mutant CFTR protein in cystic fibrosis

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CFTR stabilization at the plasma membrane (PM) represents a promising yet underexplored strategy to improve the rescue of mutant CFTR proteins implicated in cystic fibrosis (CF). At the PM, CFTR associates with numerous protein partners, which coordinate cAMP-dependent signaling crucial for CFTR regulation. Proper cytoskeletal integrity provides the necessary scaffolding to stabilize CFTR and recruit key regulatory proteins such as the cAMP sensors PKA and EPAC1 [1]. Among the cytoskeletal components in close proximity to CFTR, we identified the capping protein CAPZA2 and the inverted formin INF2 – with the first being a positive regulator and the second a negative regulator of CFTR stability at the PM [2]. Our aim here is to elucidate how the crosstalk between the cytoskeleton and cAMP signaling regulates CFTR trafficking by investigating the role of EPAC1, CAPZA2, and INF2 in CFTR trafficking and localization at the PM. Using primary nasal epithelial cell cultures from people with (pwCF), CF bronchial epithelial cells expressing wild-type or mutant CFTR, assays including Western blotting, co-immunoprecipitation, and cell surface biotinylation were performed. Live cell imaging assessed cAMP pools, and proximity labeling techniques identified INF2 interactors. Results showed that knockdown of INF2 and CAPZA2 had a particular impact in membrane-associated cAMP pools in both wild-type and F508del CFBE cells (increase for INF2 and decrease for CAPZA2). Activation of EPAC1 increased PM wt-CFTR association with NHERF1, whereas the opposite effect was observed for F508del-CFTR. INF2 lower molecular weight isoforms were detected in native cells, and ongoing interactomics studies by proximity labeling suggests an enrichment of cytoskeleton proteins among ER INF2 interactors in CFBE cells. Furthermore, a bioinformatic analysis using an MS/MS-based method ranked proteins relevant to CFTR PM stabilization and identified novel potential regulators. Together, these findings reveal the complex regulation of CFTR by EPAC1, INF2, and CAPZA2, underscoring the critical need to understand their roles in cytoskeleton-cAMP signaling crosstalk to modulate CFTR function and potentially improve CF handling.

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P 4

Revertant mutations in nucleotide binding domain 1 nearly restore the protein processing and channel gating of ΔF508-CFTR

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Protein processing and channel gating of the cystic fibrosis transmembrane conductance regulator (CFTR) Cl - channel are heavily disrupted by the cystic fibrosis mutation ΔF508, partly due to displacement of the G509 residue in the polypeptide chain. To explore the underlying mechanism, CFTR revertant mutations R1070W, G550E, R553Q, R553M, R555K were individually introduced into ΔF508-CFTR with or without the mutation G509A/V510G (A/G) that corrects the G509 displacement. All these introduced mutations showed no effect on the protein expression of matured wild-type CFTR, but mildly increased that of matured ΔF508-CFTR. Only R1070W, G550E and R555K further increased the matured protein expression of ΔF508-A/G-CFTR. Moreover, mutations G550A, G550I, G550D, and D529E also variously increased matured ΔF508-CFTR expression, suggesting that G550E and R555K mutations may enhance ΔF508-CFTR protein processing by altering local intradomain interactions. In ΔF508-A/G-CFTR, replacing G509A with G509L, G509S, G509T and G509M all increased matured protein expression. Interestingly, ΔF508-A/G-CFTR with additional mutations G550E, R555K, G509S, G509T or G509M displayed matured protein expression comparable to that of wild-type CFTR, suggesting that these combinations remarkably correct structural flaws that lead to the protein processing defect. Channel gating of these CFTR revertants was also significantly enhanced. The study of limited trypsin proteolysis indicate that protein fragments of wild-type CFTR showed an abundant band at ~90 kDa, in contrast to that of ΔF508-CFTR at ~65 kDa. Furthermore, the pattern of peptide fragment distribution for ΔF508-A/G-CFTR with G550E or G509T both were similar to that of wild-type CFTR. The data suggest that protein folding of above two revertants may be a key for correction of their protein processing and channel gating defects. Our data may suggest that abnormal intradomain interactions around residues G509, G550E and R555K are the major cause of functional defects in ΔF508-CFTR, and could be a target for future new drug discovery.

P 5

Protein and small molecule mediators of the rescue of p.Phe508del-CFTR through GRK5 inhibition

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Introduction: The standard of care for Cystic Fibrosis (CF) consists of small molecule modulator drugs which bind to and correct the fundamental molecular defects in the CFTR molecule introduced by pathogenic genetic variants. The state-of-the-art is represented by the Highly Effective Modulator Therapies (HEMTs) Kaftrio ® /Trikafta ® or Alyftrek® , both containing two potentiator molecules – which mitigate the folding, maturation, traffic and turnover stability of CFTR – and one potentiator – which maximizes the open probability of CFTR channels expressed at the plasma membrane (PM). The major challenges in CF pharmacotherapy are the development of therapies for all CFTR genotypes and optimization of HEMT. The p.Phe508del-CFTR variant stands as an example where HEMT still does not fully restore clinical efficacy endpoints of CF individuals to carrier levels, suggesting that therapeutic improvements may be achieved. Our group identified G protein-coupled receptor kinase 5 (GRK5) as a novel p.Phe508del-CFTR regulator: 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].
Objective: We aim to elucidate the composition 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) were treated with a library of siRNAs targeting 212 genes previously associated with CFTR traffic regulation by our team [2]. These loss-of-function assays were performed on cells treated with GRK5 inhibitors (CCG-273463, CCG-273441) or vehicle. The involvement of Endoplasmic Reticulum Quality Control (ERQC) checkpoints was assessed by rescreening the same siRNA panel using cells expressing p.Phe508del-CFTR genetic revertants. 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. Of these, 3 are also necessary for rescue by the CCG-273441 inhibitor, applied in combination with VX-661 and VX-445. The mechanistic involvement of these genes with CFTR maturation pathways is currently being investigated. Analysis of the metabolomic profile of cells under basal or GRK5-inhibited conditions suggested a direct involvement of the β-adrenergic pathway. Data analysis to ascertain the identities of some of the detected compounds is ongoing and might expand our understanding of this regulatory mechanism.
Conclusion: The proteins and small molecules pinpointed by our studies suggest the integration of the CFTR maturation pathways with other unexplored signalling processes in the cell. Targeting GRK5 might contribute to improving CF pharmacotherapy.

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P 6

Probing the structure of human-mouse CFTR chimeras using molecular modelling with AlphaFold3

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The single-channel properties of mouse cystic fibrosis transmembrane conductance regulator (CFTR) differ from those of human CFTR in two principal ways (1). First, mouse CFTR exhibits a lower unitary conductance. Second, mouse CFTR frequently enters and dwells for prolonged periods in a tiny sub-conductance state, whereas such openings are rare for human CFTR. To investigate the structural basis of these species-specific behaviours, we performed electrophysiological recordings on a panel of human-mouse CFTR (hmCFTR) chimeras complemented by an integrated modelling pipeline combining AlphaFold3 structural predictions and atomistic molecular dynamics (MD) simulations. The chimeras were constructed by replacing the membrane-spanning domains (MSDs) of human CFTR with the corresponding mouse sequences (hmTM1–6, hmTM7-12 and hmTM1-6:7–12) to assess the contribution of MSD1 and MSD2 to the conductance and gating phenotypes of mouse CFTR. To study the single-channel behaviour of CFTR chimeras, we transiently expressed CFTR constructs in CHO cells and studied them in excised inside-out membrane patches with a large Cl- concentration gradient ([Cl-]i, 147 mM; [Cl-]e, 10 mM) at –50 mV and 37 °C with ATP (1 mM) and PKA (75 nM) added to the intracellular solution. The three hmCFTR chimeras possessed a gating pattern intermediate between human and mouse CFTR. Both the single-channel conductance and open probability of the full open-state decreased in the rank order: human CFTR > hmTM7–12 > hmTM1–6 ≥ hmTM1–6:TM7–12 (n = 5-10). Like human CFTR, hmTM7–12 rarely transitioned to sub-conductance states, whereas hmTM1–6 sojourned to multiple sub-conductance states and hmTM1–6:TM7–12 resided preferentially in a tiny sub-conductance state closely resembling that of mouse CFTR.

To identify the structural features underlying these functional changes, we generated fifty AlphaFold3 structural models for human CFTR and the chimeras. The ATP-bound, phosphorylated human CFTR structure (PDB: 6MSM) was used as the reference for alignment. Overall, the predicted structures of all chimeras exhibited good superimposition of the transmembrane (TM) helices with both the human CFTR model and the 6MSM CFTR structure. However, notable deviations were consistently observed in the intracellular loops connecting TM4, TM5, TM7 and TM8. Because TM8 contributes to the narrow selectivity filter region of the pore and gates the channel, displacement of this helix could plausibly account for the reduced single-channel conductance and altered gating pattern observed in the chimeras. Such structural perturbations offer a potential explanation for the increased sub-conductance states observed in the chimeric channels. Together, these results demonstrate that the two MSDs are likely the primary structural determinants of the mouse sub-conductance state.

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P 7
Targeting a PI3Kγ–AKAP13–PKD1 signaling axis enhances F508del-CFTR trafficking and potentiates ETI therapy

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Background: Elexacaftor/tezacaftor/ivacaftor (ETI) therapy, the current standard of care for individuals carrying the F508del-CFTR mutation, combines 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 substantially improves lung function and clinical outcomes in patients heterozygous for F508del, it only partially restores CFTR activity and stability. Consequently, patients continue to experience mucus dysfunction, airway infection, and inflammation, highlighting the need for complementary therapeutic strategies.
Aims: Because boosting cAMP/PKA signaling promotes plasma membrane (PM) CFTR stabilization and enhances CFTR modulator efficacy, we hypothesized that the cAMP-elevating peptide previously developed in our laboratory (PI3Kγ MP; Patent No. WO/2016/103176; Ghigo et al., Sci Transl Med., 2022) could further increase F508del-CFTR PM density and stability, thereby potentiating the therapeutic benefit of ETI.
Methods: Cell-surface protein biotinylation followed by western blotting and immunogold electron microscopy were employed to assess CFTR PM density in HEK293T, 16HBE14o-, and CFBE41o- cells treated with PI3Kγ MP. Primary human bronchial epithelial (HBE) cells were also used to evaluate the functional impact of the PI3Kγ MP + ETI combination. Phosphoproteomic profiling was performed to identify signaling pathways activated by PI3Kγ MP.
Results: Combining PI3Kγ MP with ETI maximized ETI's corrective efficacy in CFBE41o- cells expressing F508del-CFTR by increasing CFTR abundance at the PM compared with ETI alone. Following six hours of cycloheximide treatment, 30% of ETI-rescued F508del-CFTR remained undegraded, whereas 67% persisted when PI3Kγ MP was added, indicating that PI3Kγ MP doubles the stability of corrected F508del-CFTR. Phosphoproteomic analysis revealed marked activation of protein kinase D1 (PKD1), a key orchestrator of protein trafficking. Notably, inhibition of PKD1 abrogated the PI3Kγ MP–mediated stabilization of F508del-CFTR, identifying PKD1 as the central effector of this pathway. Moreover, PKD1 activation required coordinated PKA and PKC signaling through A-kinase anchoring protein 13 (AKAP13/AKAP-Lbc). Finally, to validate these findings in a more physiologically relevant context, the combination of PI3Kγ MP and ETI was tested in primary HBE cells derived from five independent CF donors (three F508del/F508del and two F508del/G542X), resulting in up to a 25% increase in CFTR activity compared with ETI alone, confirming its potential as an add-on therapy.
Conclusions: This study identifies PI3Kγ and PKD1 as pivotal regulators of CFTR stability at the plasma membrane and highlights AKAP13 as a novel contributor to CFTR signaling. By enhancing F508del-CFTR trafficking and potentiating the efficacy of ETI therapy, PI3Kγ MP emerges as a promising add-on strategy for cystic fibrosis. Importantly, this compound is currently under clinical evaluation and has successfully completed Phase I, underscoring its translational potential and readiness for further therapeutic development.
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P 9

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

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Cystic fibrosis transmembrane conductance regulator (CFTR) is a channel that conducts chloride and bicarbonate ions and regulates fluid transport across cell membrane in epithelial tissues. Its gating is governed by phosphorylation and ATP binding and hydrolysis at its cytosolic nucleotide-binding domains (NBDs). CFTR possesses asymmetric ATPase sites: NBD2 is the consensus site that binds and hydrolyzes ATP, while NBD1 hosts a degenerate ATP site which lost its ability to hydrolyze ATP due to evolutionary mutations. CFTR is a unique among ABC-transporters in containing a disordered regulatory insertion (RI) – a 32-amino acid segment located in NBD1 near the ATP-binding site. The functional role of the RI remains incompletely understood. Our current work aims to elucidate the role of ATP binding in NBD1.

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 interpreted 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.

Differential scanning fluorimetry (DSF) confirmed that the W401A mutant exhibits decreased thermal stability compared 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 experiences loss of structure and ATP is entirely absent from its expected binding site of the domain. The potential effects of the stabilizing nanobody on the conformational equilibrium in the domain were excluded using the smFRET approach.

Using the hydrogen-deuterium exchange coupled to mass spectrometry (HDX-MS) approach, we identified peptides in NBD1 that exhibit reduced deuterium exchange in the ATP presence. These peptides are in close proximity within the tertiary structure and potentially play a role in NBD1 stabilization through physical contact between the α- and β-subdomains.

The triphosphate moiety and the adenine base of ATP both contribute to NBD1 stabilization, where the adenine base alone plays a role as a modulator of the conformational equilibrium within the domain.

To further elucidate the possible biological meaning of the conformational equilibrium, a single-channel activity assay on the full-length W401A mutant channel would be needed.

P 10

Investigating promiscuity of elexacaftor and GoSlo: can we better understand the mechanism of action?

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Background: K^+ channels contribute to generating the cell membrane potential. Because these channels are expressed in airway epithelia, their modulation can alter the driving force for anion secretion into the airway surface liquid (ASL), thereby affecting ASL hydration. We previously reported that GoSlo SR-5-6 (GoSlo), an activator of Large-conductance Ca^{2+}-activated K^+ (BK) channels, enhances CFTR activity across wild-type and multiple CF-causing CFTR variants overexpressed in HEK293 cells. GoSlo also exhibited a small but significant effect on F508del-CFTR biogenesis. This suggests that GoSlo can act directly on CFTR, revealing a promiscuous pharmacological profile. However, the relationship between GoSlo and the CFTR modulators elexacaftor and vanzacaftor, which also activate BK channels, remains unclear.

Methods: CFTR function was assessed using a high-content dual-fluorescence assay (YFP/mCherry) in HEK293 cells expressing wild-type CFTR. CFTR-mediated anion transport was quantified as the Area Above the Curve (AAC) from YFP fluorescence time course. Cells were treated acutely with ivacaftor (1 µM), elexacaftor (5 µM), GoSlo (10 µM), or relevant combinations.

In parallel, ASL height was measured by scanning ion-conductance microscopy (SICM) in primary human airway epithelial cultures differentiated at air–liquid interface (ALI) from homozygous F508del donors, following treatment with elexacaftor/tezacaftor/ivacaftor (ETI) alone or ETI in combination with GoSlo (10 µM).

Results: In HEK293 cells expressing wild-type CFTR, GoSlo alone increased CFTR activity compared with vehicle (AAC_{39}: 18.10 ± 0.94 RFU*s vs 15.08 ± 0.65 RFU*s), as did ivacaftor (19.97 ± 2.50 RFU*s). When GoSlo and ivacaftor were combined, CFTR activity was further increased (22.65 ± 3.13 RFU*s), consistent with additive potentiation effects. In contrast, elexacaftor alone increased AAC_{39} to 18.74 ± 0.68 RFU*s, while combined GoSlo + elexacaftor (18.12 ± 0.77 RFU*s) did not exceed the effect of either drug alone.

In primary F508del homozygous airway epithelial cultures, ETI increased ASL depth, reaching median values of 9.1, 23.3, and 42.1 µm across three independent cultures. Addition of GoSlo to ETI produced a consistent further increase in ASL depth in all three cultures, with median ASL depth values increasing to 12.9, 280.4, and 346.3 µm. All three changes were statistically significant.

Conclusion: The increase in ASL depth observed when GoSlo is applied in addition to ETI modulators is likely due to the impact of GoSlo on epithelial K^+ channels in a manner that improves anion secretion and ASL hydration. Combination experiments suggest that GoSlo and elexacaftor might bind to the same site on CFTR and both also have dual corrector/potentiator effects so their mechanisms of action on CFTR may be related. These findings support further investigation of the mechanistic interplay between BK channels and CFTR modulators.

P 11

Correction of CFTR nonsense mutations by SRI-41315 alone or combined with Alyftrek

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Therapeutic options for cystic fibrosis (CF) patients carrying CFTR nonsense mutations remain extremely limited. Our study aimed to evaluate the ability of the readthrough-inducing compound SRI-41315 to restore CFTR expression and function, alone or in combination with the vanzacaftor/tezacaftor/deutivacaftor regimen Alyftrek® .
We conducted a series of functional and biochemical assays using human bronchial epithelial cells from patients homozygous for *F508del* or compound heterozygous (*F508del/R1162X, F508del/W1282X, G542X/E585X*), CFBE41o⁻ cells expressing *F508del-CFTR* and FRT cells harboring nonsense variants (*Y122X*, *G542X*, *R553X*, *W1282X*). Cells were treated with SRI-41315 (1–3 µM), Alyftrek or both. CFTR activity was assessed using short-circuit current (Isc) measurements and whole-cell patch-clamp recordings. Protein maturation was analyzed by Western blot.
Our results show that treatment with SRI-41315 alone restored detectable CFTR-dependent chloride currents across all nonsense variants tested, demonstrating forskolin and ivacaftor/deutivacaftor responsiveness and complete inhibition by CFTRinh-172. Co-treatment with SRI-41315 and Alyftrek further increased current amplitude and promoted the appearance of the mature, fully glycosylated C-band on Western blots, indicating enhanced CFTR trafficking once full-length protein was produced by readthrough.
In conclusion, our findings identify SRI-41315 as a promising standalone or adjunct therapy to extend modulator-based therapy to class I CFTR mutations, addressing a major unmet need in cystic fibrosis treatment.

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P 12

Cystic fibrosis airway epithelium secretome profiling: new insights into CFTR-related dysregulations

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The airway epithelium contributes to host defense through a variety of mechanisms, including the secretion of a wide range of defense proteins and peptides, antioxidants, anti-proteases, growth factors, chemokines and cytokines. In cystic fibrosis (CF), there is a vicious circle of respiratory infection, exaggerated neutrophil-predominant lung inflammation, epithelial remodeling and lesions favoring mucus stasis and respiratory infection. Lung lesions appear to be largely the consequence of a failure of airway epithelial defense. It has also been suggested that airway inflammation is due to epithelial dysfunction secondary to CFTR mutations, resulting in impaired innate host defense systems. However, the link between altered innate host defense
proteins in CF surface liquid and CFTR defects has not been clearly demonstrated.

Our aim was to assess the impact of the CFTR defect on the secretome of CF bronchial epithelium, without contamination by inflammatory cells and bacterial secretions, while retaining the influence of inter-patient variability and directional epithelial secretion associated with polarization and differentiation.
Bronchial epithelial cells from CF patients (F508del/F508del; n=8) and healthy subjects (lung donors; n=8) were cultured at the air-liquid interface for 35 days until complete epithelial differentiation. The 24-hour secretions from these epithelia were collected and analyzed by quantitative liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS).
Proteome profiling identified and quantified 1629 proteins, of which 118 were found differentially abundant between non-CF and CF secretions ($\log_2$ fold change≥0.58; $p<0.05$), with 29 proteins upregulated and 89 proteins downregulated in CF secretions. Ninety-four differentially expressed proteins (80%) were classified as secreted according to the SecretomP and SignalP databases and the Human Secretome Atlas initiative. Seventy-three biological processes (Gene Ontology) were enriched. According to the Reactome database, 18 signaling pathways were enriched in differentially identified proteins, among which immune system pathways are the most enriched and represented, together with protease/antiprotease activity.
Our results show a CFTR-related dysregulation of the expression and secretion of numerous proteins, notably involved in host defense. These observations are indicative of a CF airway epithelium with a constitutive altered innate immunity, suggesting that the downstream consequences of CFTR mutation set the stage for chronic inflammation and infection in the cystic fibrosis airways.
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P 13

Intestinal CFTR-mediated chloride currents do not parallel bicarbonate secretion rates in CF

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Background: The majority of people with cystic fibrosis (pwCF) are treated with cystic fibrosis transmembrane conductance regulator (CFTR modulator drugs, mainly Elexacaftor/Tezacaftor/Ivacaftor (ETI), although gastrointestinal symptoms remain to be a burden. *In vitro* platforms which strategize individual CFTR modulator response are based on measuring CFTR-mediated anion (mainly Cl^-) secretion, but do not assess HCO_3^- secretion. However, both CFTR-mediated chloride (Cl^-) and bicarbonate (HCO_3^-) conductance are important to regulate fluid secretion/absorption and mucus homeostasis alongside other apical channels/transporters. Intestinal HCO_3^- secretion occurs through CFTR, directly and in collaboration with DRA (SLC26A3), a Cl^-/HCO_3^- exchanger. We aimed to elucidate HCO_3^- secretion and its response to ETI to interrogate failed clinical ETI response in the CF intestine.

Objective: To simultaneously assess CFTR-mediated Cl^- and HCO_3^- secretion in human intestinal organoids (HIO) from pwCF and their response to CFTR modulator treatment.

Methods: HIO were generated from rectal biopsies of healthy controls (HC, n=2), and 14 pwCF classified as: G551D/other (G551D HIO, n=3), F508del/F508del (DF HIO, n=3) and nonsense mutations (stop HIO, n=8). In 2D HIO monolayers, transepithelial anion current (I_{eq}, μA/cm^2) and HCO_3^-secretion rate (J_{HCO3}, μEq/min/cm^2) were measured in parallel using a pH-stat linked to Ussing chamber equipment. Forskolin (Fsk) was applied to stimulate CFTR. G551D HIO were treated with ivacaftor; DF HIO were treated with ETI; and stop HIO were treated with ETI plus G418, a read-through agent.

Results: In HC HIO J_{HCO3}-Fsk (0.51±0.18 μEq/min/cm^2) was reduced with pharmacological inhibition of CFTR (0.22±0.18 μEq/min/cm^2, p=0.003) and DRA (0.34±0.08 μEq/min/cm^2, p=0.27), and under chloride-free conditions (0.30±0.23 μEq/min/cm^2, p=0.04), where Cl^-/HCO_3^-exchange is excluded. These results support a shared role for CFTR and DRA in HCO_3^- secretion.

In contrast to diminished I_{eq}-Fsk, CF HIO show residual J_{HCO3}-Fsk not significantly different from HC but still reflective of genotype severity with G551D>DF>stop HIO. In G551D HIO ivacaftor treatment slightly increased I_{eq}-Fsk (-18.16±12.74 to -30.56±20.51 μA/cm^2, p=0.28) and J_{HCO3}-Fsk (0.77±0.53 to 1.06±0.96 μEq/min/cm^2, p=0.58). In DF HIO ETI treatment increased I_{eq}-Fsk (-0.70±0.68 to -25.98±15.12 μA/cm^2, p=0.002), yet J_{HCO3}-Fsk remained unchanged (0.43±0.25 to 0.50±0.25 μEq/min/cm^2, p=0.62). Finally, in stop HIO ETI+G418 treatment increased I_{eq}-Fsk (-0.55±0.66 to -8.97±10.72 μA/cm^2, p=0.026) and J_{HCO3}-Fsk (0.31±0.32 to 0.61±0.38 μEq/min/cm^2, p=0.089).

Conclusion: Despite CFTR modulator-mediated increases in anion currents, the response of HCO_3^- secretion depends on CFTR mutation. Unlike G551D and stop HIO, HCO_3^- secretion in F508del HIO is unchanged with CFTR modulator treatment. This lack of response *in vitro* may explain the lack of clinical response in the CF intestine. Given the involvement of CFTR and DRA in healthy control HCO_3^- secretion, future studies will be directed to elucidate residual J_{HCO3}-Fsk function seen in CF HIO.

P 14
Airway HCO_3^- and H^+ secretion: role of pulmonary ionocytes

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Background: Alkalinization of the airway surface liquid (ASL) during inhalation is bicarbonate-dependent, antimicrobial, and diminished in CF[1], however the steady-state regulation of ASL [HCO_3^-] remains poorly understood[2]. Ionocytes have high CFTR and V-ATPase expression and are thus implicated in both HCO_3^- and H^+ secretion. Pendrin-mediated HCO_3^- secretion is increased during inflammation[3,4] and may be counteracted by H^+ secretion via ATP12A (H^+/K^+-ATPase)[5], which is upregulated in CF[6].

Aim: To explore ASL acid/base homeostasis and the role of ionocytes.

Methods: Ionocyte-enriched cultures were prepared by transducing primary HBE cells with FOXI1+EGFP adenovirus[7] and compared with control (EGFP transduced), naïve (untransduced), ionocyte ablated, and inert cultures (paraformaldehyde fixed) cultures. The pH of artificial ASL (30 µL) containing a range of HCO_3^- concentrations was monitored by imaging SNARF-1 dextran fluorescence while the ASL was exposed to room air or 5% CO_2 to mimic inhalation or exhalation, respectively. Cultures were perfused basolaterally with 25 mM HCO_3^-/5% CO_2. Net acid secretion was determined using a carbonic acid reaction network model that corrects for instantaneous changes in HCO_3^- buffering. Net base secretion was monitored as the increase in ASL pH during exposure to apical 5% CO_2.

Results: pH gradually declined in ASL containing HCO_3^- and this acidification was inhibited >98 % by apical ouabain (1 mM) or K^+ removal. Similar acidification by FOXI1-transduced and control cultures suggested little, if any, ATP12A activity was localized in ionocytes. cAMP caused a transient (<10 min) increase in acid secretion rate that was most pronounced in FOXI1-transduced cultures. cAMP stimulated H^+ efflux was abolished by bafilomycin-A1 or $CFTR_{inh}$172, suggesting it results from electrogenic V-ATPase activity in ionocytes and requires CFTR channel function, most likely as a Cl^- shunt pathway. ASL pH remained constant at ~6.1 with 5% CO_2 and 2.5 mM [HCO_3^-] and was increased by cAMP in FOXI1-transduced cultures, providing further support for HCO_3^- secretion by ionocytes[8]. $CFTR_{inh}$-172 inhibited this alkalinization, consistent with the inability of CF cells to alkalinize ASL[5].

Conclusions: cAMP stimulates H^+ and HCO_3^- secretion by pulmonary ionocytes through activation of V-ATPase and CFTR. These fluxes are superimposed on constitutive acid secretion that is mediated by ATP12A mainly in non-ionocytes.

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P 15

Impact of low molecular weight hyaluronic acid on airway epithelial remodelling in cystic fibrosis

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Background: Remodelling of the airway epithelium, with increased epithelium height, hyperplasia of basal and secretory cells, and alteration in the number of ciliated cells, is hallmark of cystic fibrosis (CF) and amplifies the defect in mucociliary clearance in patients with CF (pwCF). Hyaluronic acid (HA) is a natural high molecular weight (MW) polymer that can be fragmented into lower MW in injury or inflammation context. Its biological effects can vary depending on its molecular size and the receptors involved.

Aims: The aim of this study was to determine the ability of low-MW-HA (LMW-HA; 40 kDa) to prevent epithelial remodelling and improve ciliated cell differentiation and/or cilia function, in order to determine its potential therapeutic use for pwCF.

Methods: Bronchial epithelial cells from pwCF (F508del/F508del) were cultured in an air-liquid interface (ALI) and treated or not with HA of different MW (40kDa, 500kDa, 1.4MDa) and concentrations (0.5 mg/ml, 1 mg/ml, 2 mg/ml). Epithelium height was determined after hematoxylin/eosin/saffron staining. The impact of HA on the representation of different cell populations (ciliated, basal and secretory cells) was quantified after immunofluorescence detection. The expression of HA receptors was analyzed by western blot. Ciliary function was assessed using videomicroscopy by measuring the ciliary beat frequency (CBF) in cultures treated or not with LMW-HA, with a chronic or acute treatment with *P.aeruginosa* lipopolysaccharides to mimic bacterial infection, or with Poly I:C to mimic viral infection. Total RNAs were extracted at different time point during the regeneration process from cultures treated or not with LMW-HA, and gene expression was determined by bulk RNA sequencing.

Results: Our results showed that LMW-HA induced a significant decrease in CF epithelium height at 1 mg/ml (-17%) and 2 mg/ml (-10%), compared to untreated cultures. LMW-HA treatment was the only one HA to significantly enhance ciliated cell differentiation, in a dose-dependent manner (from +150% at 0.5 mg/ml to +220% at 2 mg/ml). At 2 mg/ml, LMW-HA reduced the number of basal (-14%) and secretory cells (-75% for Muc5AC+ and -63% for Muc5B+ cells), and modulated CD44 expression, whereas the RHAMM expression was not modified. We also demonstrated that LMW-HA increased CBF (+140%).
Combined with Poly I:C acute treatment, LMW-HA also increased CBF (+7%).

Finally, RNAseq analysis showed that 290 genes were differentially expressed between control and LMW-HA treated cultures at ALI day 25, with 148 up-regulated and 142 down-regulated, among them interleukins such as IL-6 and IL-8, and gel-forming mucins Muc5B and Muc5AC which were down-regulated.

Conclusions: Our results show that LMW-HA prevents CF bronchial epithelial remodelling and improves ciliary beat frequency.

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P 16

Investigating TMEM16A and TRPV4 as calcium-signaling-related proteins involved in airway hydration and defense mechanisms in cystic fibrosis

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Background and rationale: Loss-of-function of the CFTR chloride channel impairs mucociliary clearance (MCC), causing cystic fibrosis (CF) lung disease. Alternative targets, such as the calcium-activated TMEM16A chloride channel, are considered for the treatment of patients carrying undruggable CFTR mutations. However, the best approach to target TMEM16A in CF, whether with potentiators or inhibitors, is a matter of debate. Another interesting protein is the TRPV4 calcium channel. TRPV4 is a potential sensor of mechanical and chemical stimuli, involved in the response to pathogens and linked to CFTR activity.

Hypothesis and objectives: Our hypothesis is that TMEM16A and TRPV4 could be therapeutic targets in CF. Our goal is to clarify their role in the airways to modulate them in the most appropriate way. For TMEM16A, we used the CRISPR/Cas9 strategy to knockout the gene in airway epithelial cells and evaluate the consequences on airway surface properties. Regarding TRPV4, we are investigating the mechanisms linking this channel to CFTR and to calcium-activated dual oxidases that produce H_2O_2 as a bactericidal agent.

Essential methods: We are using differentiated human bronchial epithelia (HBE) in which TMEM16A and TRPV4 function are altered with genetic and/or pharmacological approaches. We carried out a panel of experiments to evaluate intracellular calcium mobilization. We are evaluating the airway surface liquid properties, mucin secretion, epithelial composition/morphology, and H_2O_2 production

Results: TMEM16A-defective epithelia, generated by nucleofection of basal stem cells, showed a near total ablation of calcium-dependent chloride secretion without alteration of calcium signaling. Pharmacological TRPV4 activation led to release of ATP which then activated CFTR through stimulation of adenosine and purinergic receptors. Bacterial supernatants elicited a significant calcium increase possibly through TRPV4.

Conclusions: The results obtained so far show that we are able to ablate TMEM16A function in differentiated epithelia which will allow us to assess its physiological role. TRPV4 appears to regulate H_2O_2 and ATP release. It will be important now to determine how pharmacological modulation of TMEM16A and TRPV4 leads to improvements in airway surface hydration and innate defense mechanisms of the airway epithelium.

P 17

Investigation of CFTR function and epithelial barrier properties at single cell resolution using multi-electrode array technology

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To date, most CFTR-targeting studies have focused on their effect on CFTR mediated ion transport and hydration in the airway epithelium. Although it remains unknown if CFTR modulator treatment acts similarly in all CFTR expressing cells within an epithelium, this is unlikely the case for next-generation treatments like gene replacement or gene editing. Current assays lack the required high spatial resolution and/or throughput to study potential differences in rescue of CFTR function between cells in a label-free manner. We therefore set out to investigate the effect of CFTR treatments on CFTR ion channel function and barrier integrity in individual epithelial cells using imec's high density multi-electrode array (MEA) chip.

CFTR ion channel function and local barrier integrity were studied using electrical impedance recordings on MEA chips with >16.000 subcellularly sized (8µm) electrodes. For initial studies, we used HEK293T cells, the intestinal colon cancer cell line CaCo2, and 16HBE to represent increasing model complexity. Cells were grown on coated chips. Impedance was recorded at 1kHZ before and after 20 minute incubation with CFTR activator forskolin (10µM) or forskolin+CFTRinh-172 (50µM). CFTR function was determined as the % change. Barrier integrity was determined using the first recording in addition to immunocytochemistry for tight junction protein ZO-1 and measuring permeability with FITC-dextran (4/20 kDa). In parallel, we recorded CFTR function by transepithelial electrical resistance (TEER) on transwell-grown cultures.

As proof of concept, we used HEK293T, CaCo2 and 16HBE cells to measure CFTR function with high spatial resolution by impedance recordings. In HEK293T cells, we could show a significant restoration of F508del-CFTR function upon elexacaftor/tezacaftor/ivacaftor treatment ($p=0.0087$). We observed a CFTR-specific impedance drop of ~20% upon CFTR stimulation by forskolin in CaCo2 cells (vs. ~3% in mock treated controls; $p=0.0003$), which was completely inhibited by co-incubation with Inh-172 (~1% drop; $p=0.0047$; vs. mock: $p=0.96$), while tight junctions were unaffected. In preliminary experiments in 16HBE, CFTR activation and inhibition resulted in ~4% decrease and ~8,5% increase in impedance, respectively. In 16HBEgeG542X, both treatments increased impedance by ~1%, showing again the specificity for CFTR. We are currently optimizing the growth of primary cells on the platform, including rectal organoid-derived monolayers and submerged differentiated airway epithelia. For the latter we could successfully follow-up growth and differentiation through impedance for over 50 days.
The use of high-density electrical impedance recordings allows to study simultaneously barrier integrity and CFTR channel function at single cell resolution. We currently show CFTR function in CF-relevant, but laboratory, models and are in the process of optimizing the assay to primary cell models. Next, we aim to correlate this single cell CFTR rescue with CFTR protein expression. We envision that this novel model will contribute to unravelling the full effects of CFTR-targeted therapies.

P 18

A secretory cell is never late: CFTR emerges early in epithelial regeneration

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Introduction: In people with cystic fibrosis (pwCF), chronic infection and inflammation lead to repeated cycles of airway epithelial injury and repair. Current knowledge of CFTR function comes from mature, well-differentiated airway epithelial cultures, where ionocytes and secretory cells show the highest CFTR activity. However, epithelial regeneration is dominated by transitional secretory cells, and whether these early cell states contribute to CFTR function remains unknown. We therefore examined CFTR expression and activity during early differentiation to determine whether transitional secretory-lineage cells play a functional role.

Methods: Human nasal epithelial cells (HNECs) from non-CF subjects (n=3) were differentiated in air-liquid interface (ALI) cultures. Mucociliary differentiation was characterized through immunofluorescence and qPCR for secretory and ciliated markers on days 0, 7, 14, and 21. At the same time points CFTR-dependent chloride transport was assessed using Ussing chamber recordings. CFTR function during early differentiation was furthermore evaluated in day-7 monolayer-derived airway organoids from pwCF (F508/delF508del; n=2) using the Forskolin induced swelling (FIS) assay. Finally, CFTR modulator (ETI) responses were quantified using the FIS assay in secretory-enriched vs ciliated-enriched organoids, representing early vs late differentiation states, respectively.

Results: Investigation of mucociliary kinetics revealed a significant increase in MUC5AC+ secretory cells at day 7, followed by a decline at day 14 and 21, coinciding with an increase in β-tubulin IV+ ciliated cells at day 14 and 21. qPCR analyses of MUC5AC, CFTR, and the goblet-cell regulator SPDEF supported these observations. In addition, CFTR-dependent ion transport in Ussing chambers showed a comparable temporal profile. Organoids from early differentiated airway monolayers of pwCF formed cystic structures containing MUC5AC+ cells and displayed ETI responses in FIS assays. Furthermore, ETI responses were significantly higher in secretory-cell-enriched organoids compared to ciliated-cell–enriched organoids, consistent with the early secretory dominance during regeneration.

Conclusion: We show the early regenerative airway epithelia rapidly adopt a secretory-dominant state that transitions towards ciliated cells over time.

These transitional-secretory airway epithelia display high CFTR function that declines as differentiation progresses, suggesting a previously unrecognized role for early secretory lineages in epithelial regeneration and possibly CF-related repair defects

P 19

Identification of novel SLC26A9 inhibitors for the study of non-CFTR mediated anion secretion

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Background: Enhancing non CFTR-mediated anion secretion is a mutation-agnostic therapeutic approach for the treatment of individuals with cystic fibrosis (CF) with two null CFTR alleles without approved CFTR modulators. Human genetic studies identified *SLC26A9* as a modifier of CF lung, pancreatic and intestinal phenotypes. SLC26A9 is an epithelial chloride transporter that contributes to epithelial surface hydration and interacts with CFTR. However, the contribution of SLC26A9 to ion transport is poorly understood, in part, due to its intertwined activity with CFTR. While a SLC26A9 inhibitor S9-A13 was recently used to separate the contributions of SLC26A9 and CFTR to anion secretion, S9-A13 showed an effect on CFTR currents at high concentration and it failed to inhibit constitutive ion transport in mouse trachea despite clear expression of SLC26A9. Thus, additional SLC26A9 inhibitors would be a useful complement to investigations aimed at disentangling the SLC26A9 role in epithelial ion transport, which we aimed to identify here.

Methods: To identify SLC26A9 inhibitors, we carried out a high-throughput screen (HTS) of 104,280 chemically diverse compounds using a fluorescent cell-based assay. This assay measures membrane potential (MP) to monitor ion transport in GripTite cells with inducible expression of human SLC26A9.

Candidate inhibitors were selected based on reduced MP responses, significant dose-response curves and drug-like properties. After the primary screen, we examined the hit compounds in cells with and without SLC26A9 expression. Inhibitors were prioritized based on (I) the significant inhibition at 1X IC_{50} concentration in the GripTite cells with SLC26A9 expression, (II) no off-target effect in counter assays using the GripTite cells without SLC26A9 expression, and (III) having low IC_{50} values. Specificity of the prioritized inhibitors was investigated using RNAi knockdown against SLC26A9.

Results: The HTS was robust with a mean plate Z’ value of 0.65 and mean signal to baseline ratio of 2.65. Twenty-one compounds were selected as candidate SLC26A9 inhibitors in the primary screen. Following careful examination of repurchased hit compounds, five inhibitors were prioritized based on the prioritization plan outlined in *methods*. The prioritized inhibitors indicated specificity for SLC26A9 in knockdown experiments. Comparing the prioritized inhibitors with S9-A13 using the fluorescent MP assay showed a similar reduction of SLC26A9 response to S9-A13. While our inhibitors have higher IC_{50} values compared to S9-A13, chemical derivatization of these hit compounds is yet to be carried out and will improve their potency.

Conclusion: Selectivity testing of the prioritized inhibitors against CFTR, MP assay testing in lung and pancreatic cells with endogenous *SLC26A9* expression and comparison to S9-A13 using Ussing experiments are in progress. These experiments will demonstrate the relative benefits of the different tools for isolating and determining the contribution of SLC26A9 to chloride transport. The two most potent and selective inhibitors will be optimized for potency

P 20

Pediatric cystic fibrosis with CFRD and celiac disease: contribution of TCF7L2 rs7903146 (TT) and rs4077468 (GG) variants in disease complexity

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Background: Cystic fibrosis–related diabetes (CFRD) is a common metabolic complication in adolescents with cystic fibrosis (CF). Genetic modifiers, including variants in TCF7L2 and SLC26A9, have been associated with increased CFRD susceptibility. The coexistence of CF, CFRD, and celiac disease in childhood is uncommon and presents additional diagnostic and therapeutic challenges.

Case presentation: We report a 14-year-old female with CF carrying compound heterozygous CFTR mutations (p.Glu831Ter and p.Ser573Phe). Sweat chloride values were borderline (36.4 and 31.0 mmol/L), but her clinical features were consistent with CF. At age 7 years, she was diagnosed with celiac disease based on markedly elevated anti-tTG IgA (297 U/mL) and IgG (22.6 U/mL) and initiated a gluten-free diet. At age 12.4 years, she presented with diabetic ketoacidosis and was diagnosed with CFRD, requiring initiation of insulin therapy. Genotyping identified high-risk diabetes-associated and CF-modifie variants: rs7903146 TT (TCF7L2) and rs4077468 GG (SLC26A9); rs838440 in PTMA was non-risk. At follow-up, she demonstrated favorable growth (height z-score 2.03; BMI z-score 0.43) and preserved pulmonary function (FEV_1 and FVC approximately −1.0 z-score).

Conclusion: This case underscores the interplay between CF, autoimmunity, and genetic predisposition in the early onset of CFRD. Incorporating genetic profiling may enhance risk assessment and individualized management strategies for CF patients with complex endocrine or autoimmune manifestations.

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P 21

Adipogenesis, a key factor affecting bone health in patients with cystic fibrosis

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Cystic Fibrosis-related Bone Disease (CFBD) affects 52% of adult CF patients. A relationship exists between low bone density and increased medullar adiposity, (Rozman, 1989; Verma, 2002; Zhou, 2008; Piccinin, 2014). Osteoblasts obtained from CF induced pluripotent stem cells (iPSCs) evidenced an altered phenotype with a higher *PPARG* level (adipocyte transcription factor) (Dumortier, 2025). We hypothesized that, skeletal stem cells (SSCs), common precursor for both adipocytes and osteoblasts, exhibit a preferential commitment toward adipocyte differentiation when carrying *CFTR* mutations.
SSCs from non-CF donors (n=7) have been differentiated into adipocytes and osteoblasts during 21 days, with or without CFTR pharmacological inhibitors (Inh_{172} or BPO-27). Oil Red'O and Bodipy^{TM} stainings, and adipocytes specific proteins by immunostainings like FABP4 and PPARG were performed. MicroCT analysis was performed in CF mice in both aging (up to one year) and ovariectomy (OVX) models. These studies enabled the characterization of bone microarchitecture and medullary adiposity.
CFTR blockade spontaneously induces lipid droplets formation in osteoblast and undifferentiated cultures with both inhibitors. In adipocyte cultures, the mutation increases the number and the size of adipocytes. This may reflect a prefered adipocyte commitment due to impaired CFTR function. CF OVX mice exhibited a markedly worsened bone phenotype, characterized by reduced trabecular number and thickness, lower bone mineral density, and bone volume, along with increased trabecular separation, compared with non-CF OVX mice and wild type littermates. In aged mice, male CF mice have a reduced lifespan, while female CF mice show accelerated bone decline. Bone marrow adiposity is still under study, but preliminary data indicate elevated bone tissue adiposity in the CF genotype.
These results indicate that pharmacological inhibition of CFTR in SSCs induces a preferential orientation towards the adipogenesis pathway at the detriment of osteoblastogenesis, evidencing a link between CFTR dysfunction and bone adipocytes in CFBD.
Data showing that aging in both sexes and postmenopause-induced osteoporosis accelerate bone disease in CF, highlights the need for adapted treatment for CFBD in the aging CF population. Finally, understanding the role of lipid metabolism in bone, in this context, may represent a promising avenue to better understand and treat CFBD.

P 22
The control of airway surface liquid pH in cystic fibrosis (CF) and non-CF airways

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We are investigating factors that contribute to the control of airway surface liquid (ASL) pH. ASL produced by the airway's surface epithelium and submucosal glands acts as a host defense to trap and remove inhaled pathogens. Its pH regulation via natural buffers and the transepithelial movement of H^+ and HCO_3^-, plays an important role in many lung diseases. During tidal breathing, CO_2 variation in the airways causes pH oscillations that are thought to be dampened by mucins. This has been suggested to increase the bacterial killing power of the ASL. We have examined ASL pH in vitro using a chamber equipped with a scanning ion conductance microscope. We used a nanopipette probe as a H^+-selective electrode for direct measurement of changes in ASL pH. In this approach no disturbance of the ASL (e.g. adding excess fluid or dyes) is required. We are thus able to have real-time monitoring for ASL pH with minimal chemical or physical disturbance to the ASL microenvironment. The humidified CO_2/air mixture in the chamber is controlled by a built-in CO_2 sensor. The CO_2 level then oscillates between 0.1% and 5% resembling the changes of that occur during respiration. We find CF cultures grown in Pneumacult medium generally have an acidic ASL pH (median 5.70) and may have a more acidic ASL than that of non-CF cultures (median 6.65) in 5% CO_2. We also find that the ASL of cultures grown in Pneumacult medium undergo relatively small oscillations in response to CO_2 changes (median 0.05 for CF and 0.16 for non-CF). ETI treatment did not substantially alter the ASL pH in 5% CO_2 (median 5.70 for untreated vs 5.77 for treated). Further, although ETI did increase the median oscillation size (median 0.05 for untreated and 0.13 for treated), this effect did not reach statistical significance. The relative acidity of the ASL has important implications for buffering and we are examining this by using a novel CO_2oscillation approach.

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Elexacaftor/Tezacaftor/Ivacaftor (ETI) restores bicarbonate transport of F508Del-CFTR

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Background: Cystic fibrosis (CF) arises from dysfunction of the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR), an epithelial ion channel that maintains airway surface homeostasis by regulating chloride (Cl^-) and bicarbonate (HCO_3^-) transport into the airway surface liquid. The triple modulator combination Elexacaftor/Tezacaftor/Ivacaftor (ETI) markedly improves lung function in people with CF (pwCF) who carry at least one F508del-CFTR allele.

While ETI-mediated rescue of Cl^- transport is well established, its effects on transepithelial HCO_3^- secretion remain poorly characterized. To address this gap, we investigated CFTR correction by directly comparing CFTR-mediated HCO_3^- and Cl^- transport following F508del-CFTR rescue in primary human nasal epithelial cells (HNECs). We additionally examined modulator responsiveness of the rare CFTR allele R334W. R334W is a class IV missense mutation located in TM6 of MSD1 and associated with minimal CFTR activity. Previous studies indicate that R334W reduces CFTR single-channel conductance by ~60%. In CF bronchial epithelial cell lines models, the mutant protein appears to be normally processed but displays very low channel activity. Data on this genotype are limited but suggest that it responds poorly to ETI.

Methods: HNECs from pwCF with at least one F508del allele were treated with ETI for 48 hours. CFTR-dependent HCO_3^- and Cl^- currents were quantified using short-circuit current (Isc) recordings in Cl^--free or HCO_3^--free buffers after CFTR activation with forskolin. The same assays were performed using HNECs from a patient with the genotype M1T/R334W. M1T is a true class I variant that produces no CFTR protein and is non-responsive to ETI, enabling isolation of R334W-mediated function. For the M1T/R334W HNECs, additional modulator combinations were tested - VX-121 (Vanzacaftor)+VX-770, VX-445+VX-770, VX-661+VX-770, and VX-121+VX-661+VX-770.

Results: ETI increased F508del-CFTR–dependent HCO_3^- transport to ~32% of wild-type (WT) levels and F508del-CFTR–dependent Cl^- transport to ~66% of WT in HNECs.

In R334W-CFTR, baseline (DMSO) Cl^- transport was ~4% of WT. VTI increased Cl^- transport to ~8% of WT. ETI further increased R334W-CFTR–dependent Cl^- transport to ~20% of WT. No other modulator combination produced an improvement above baseline. Next steps will involve testing additional potentiators and performing the same set of experiments to quantify R334W-mediated HCO_3^- transport.

Conclusions: ETI partially restores both F508del- and R334W-CFTR function, although R334W shows limited responsiveness compared with F508del. These results highlight the importance of directly measuring ion-specific CFTR function to guide personalised modulator therapy for rare CFTR alleles.

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Rescue of CFTR function by elexacaftor-tezacaftor-ivacaftor in F508del-CFTR biliary epithelium

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Objectives: Cystic fibrosis (CF) is associated with abnormal biochemical liver tests, cholestasis, biliary inflammation, and liver fibrosis. We investigated the effects of the CFTR modulator combination elexacaftor-tezacaftor-ivacaftor (ETI) on fluid secretion and pH regulation in CF biliary epithelium.

Methods: Intrahepatic cholangiocyte organoids (ICOs) were cultured from CF (F508del-CFTR) explant liver tissue of transplant recipients, and ICO-derived cells were used to culture epithelial monolayers on a permeable substrate. ICO swelling upon exposure to the cAMP agonist forskolin was monitored using confocal microscopy. A ratiometric fluorescent probe (SNARF) was used to assess the luminal surface pH of monolayers. Luminal mucus accretion on monolayers was monitored by tracking of fluorescent nanobeads.

Results: ETI enhanced forskolin-dependent swelling in F508del-CFTR ICOs, indicting rescue of mutant CFTR function. Forskolin treatment led to luminal alkalization in both CF and non-CF biliary epithelial monolayers. Forskolin also stimulated mucus release, independent of CFTR genotype. Pharmacological inhibition of CFTR did not attenuate the forskolin-dependent pH response of control monolayers, and ETI did not enhance luminal alkalization in CF epithelia. Removal of luminal chloride, which blocks chloride-bicarbonate exchanger AE2 (*SLC4A2*) activity, strongly attenuated forskolin-dependent luminal alkalization in CF-, but not in control epithelia.

Conclusions: ETI restores cAMP-dependent fluid secretion in F508del-CFTR-expressing biliary epithelium. Forskolin/cAMP stimulates luminal alkalization in CF biliary epithelium, independent of CFTR, conceivably by promoting bicarbonate secretion through AE2. In non-CF biliary epithelium, both CFTR and AE2 appear to mediate bicarbonate secretion. In CF biliary epithelium, the loss of such redundancy may be involved in the development of cholangiopathy.

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P 25

Linking CFTR dysfunction to EMT and cancer susceptibility in patient-derived intestinal organoids

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Background: Emerging studies report that colorectal cancer (CRC) incidence is substantially increased (~5-fold) and occurs at an earlier age in people with Cystic Fibrosis (pwCF). Furthermore, defective CFTR function has been associated with epithelial-to-mesenchymal transition (EMT), a key process in cancer progression. Our previous findings suggest that the EMT phenotype is dependent on the specific CFTR variant. We showed that the loss of ion transport alone (p.Gly551Asp, class III) is sufficient to trigger EMT, whereas the absence of plasma membrane-localized CFTR (p.Phe508del, class II) further worsens this phenotype.
Objective: To investigate how CFTR dysfunction influences epithelial differentiation and promotes EMT and cancer-associated processes in the intestine.

Methods: Intestinal organoids (IOs) generated from CF and non-CF donors were evaluated for epithelial and mesenchymal markers and cell-type composition by immunofluorescence and Western blot. CRISPR/Cas9 gene editing was used to introduce p.Phe508del and p.Gly551Asp in homozygosity in non-CF IOs to generate two novel variant-specific isogenic IO models.
Results: Both CF and non-CF IOs differentiated into diverse epithelial lineages, such as, goblet and enteroendocrine cells. Interestingly, CF IOs showed reduced expression of the epithelial marker cytokeratin 18 (CK18). Gene-edited IOs are under clonal selection and will allow direct functional testing of these CFTR variants.
Conclusion: CFTR dysfunction alters epithelial differentiation, as reflected by reduced CK18 expression in CF organoids. The newly generated isogenic IO models will provide a valuable platform to investigate how specific CFTR variants influence EMT and cancer-related pathways.

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R-vanzacaftor rescues mucociliary dysfunction in CFBE cells and a CF-like sheep model

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Objectives: Modulators targeting mutant cystic fibrosis transmembrane conductance regulator (CFTR) have transformed treatment for nearly 90% of individuals with cystic fibrosis (pwCF). However, pwCF who carry two minimal-function variants or other variants unresponsive to modulators and pwCF who do not tolerate modulators still face limited therapeutic options. Alternative ion channels that could compensate for CFTR dysfunction represent promising targets for CF airway disease. Apical chloride secretion via CFTR or TMEM16A is facilitated by an apical loop current where apical potassium efflux facilitates chloride conductance, thereby supporting fluid secretion onto the airway surface. Recently, we have shown the beneficial effects of potentiating the large conductance, calcium-activated potassium (BK) channel using basolateral R-vanzacaftor *in vitro*. Our hypothesis was that these effects could be further enhanced if R-vanzacaftor were nebulized *in vitro* and *in vivo* to achieve higher concentrations.

Methods: Primary cystic fibrosis nasal (CFNE) and bronchial epithelial (CFBE) or ovine airway epithelial cells were cultured at the air-liquid interface and allowed to differentiate for at least 3-4 weeks. Some CFBE cells were transduced with BK-knockdown (BKKD) lentiviruses as previously described. All assays were performed either by acute activation or long-term (24h and 5 days) potentiation of BK using vanzacaftor enantiomers (R and S). BK conductance was measured in Ussing chambers as short-circuit currents. Mucociliary transport (MCT) using fluorescent microbeads were assessed as published. Levels of vanzacaftor were quantified by LC-MS/MS from sheep blood and patient blood of pwCF on Alyftrek™ (that contains S-vanzacaftor). Sheep experiments to record tracheal mucus velocity (TMV) were executed as described before, using a 2 mg total nebulizer dose of vanzacaftor. Tracheal secretions and blood were collected and mucus solids and TGF-β1 assessed as published.

Results: Compared to S-vanzacaftor and control, R-vanzacaftor significantly potentiated BK in CFNE. Potentiation was not subject to desensitization over 5 days in CFBE. Comparing R-vanzacaftor in media and apical delivery by nebulization, aerosol delivery significantly increased BK potentiation and MCT. In BKKD, BK potentiation was significantly rescued by nebulized R-vanzacaftor. As seen in CFBE, ovine cells also showed acute activation by S- and R-vanzacaftor but long-term potentiation only by R-vanzacaftor. Using an LC-MS/MS assay that can distinguish the two enantiomers, showed no conversion into each other. In sheep, nebulizing a single 2 mg dose of R-vanzacaftor, showed low uptake into the systemic circulation but significantly rescued mucociliary dysfunction measured by TMV.

Conclusions: Our data show that enhancing apical loop currents by potentiating BK in CF models without modulating mutant CFTR can meaningfully and thus therapeutically enhance mucociliary function. Nebulized delivery of R-vanzacaftor shows no desensitization of BK potentiation in cellular models. Finally, single nebulized doses of R-vanzacaftor significantly rescued mucociliary dysfunction *in vitro* and *in-vivo* with no noticeable systemic side effects.

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CFTR correction, as a new therapeutic option in the treatment of chronic pancreatitis

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Introduction: Chronic pancreatitis (CP) is a progressive inflammatory disease accompanied by functional and morphological damage of the pancreas. Beside The physiological and pathophysiological relevance of pancreatic ductal epithelial cells (PDEC) it has been also demonstrated that CFTR, the main component of pancreatic ductal secretion, trafficking to the membrane is impaired in CP, resulting in a decrease HCO3- concentrations in pancreatic juice. These observations suggest that HCO3- and fluid secretion by PDEC, and mainly the CFTR channel, may play a fundamental role in the pathogenesis of CP. CFTR modulator therapy has been successfully used in the treatment of patients with cystic fibrosis, but its effect on the development and progression of CP has not yet been investigated. Therefore the aim of this study is to investigate the effect of Trikafta, on the development and progression of CP.
Methods: Human pancreatic organoids (HPO-s) were generated from patients with CP, mouse pancreatic organoids (MPO-s) were generated from animals after the induction of CP. The effect of 48hour incubation of Trikafta on organoids was assessed by microfluorimetric measurements by an inverted fluorescent microscope. Polarized expression of channels was assessed by immunofluorescent labeling (IF).
Results: Fluorescent measurements revealed significantly lower CFTR activity in CP groups compared to control organoids, which reduction was demolished by Trikafta treatment in both HPOs and MPOs. Fluorescent Na2+ measurements in HPOs have shown elevated ENAC activity compared to control organoids, which effect was significantly lowered by Trikafta. Immunofluorescent labeling of HPOs show the reduction of CFTR in the apical membrane in CP groups compared to control, while Trikafta appears to push CFTR back to the cell surface. ENAC on the other hand shows more prominent labeling in CP groups compared to control and Trikafta treated groups.
Conclusion: Our results suggest that Trikafta bares beneficial effects with marked improvement in both CFTR channel function and cell integrity, thus may contribute to the development of specific therapies for CP by targeting the restoration of CFTR function.

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IL-17A and IL-17F differentially modify ion transport and apical surface properties in human bronchial epithelia

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The airway epithelium deploys innate defense mechanisms that have a key protective role against pathogens. The mucociliary clearance (MCC) process is controlled by a fine balance of the airway surface properties. In cystic fibrosis (CF), the absence of chloride secretion through CFTR leads to dehydration of the airway surface, MCC impairment, bacterial colonization, persistent inflammation and progressive lung damage. Patients carrying F508del, the most frequent CF-causing mutation, can now benefit from highly effective modulators: VX-661 and VX-445 correctors combined with the VX-770 potentiator, known as ETI therapy. Despite improved CFTR function, airway inflammation often persists. IL-17 cytokines, particularly IL-17A and IL-17F, are involved in the immune response to extracellular bacteria and the recruitment of neutrophils in CF lung disease. These cytokines interact with IL-17 receptors, IL-17RA and IL-17RC, that can form both homodimers and heterodimers with different affinity for the two cytokines. In particular, IL-17A binds preferentially the RA-RA homodimer, while IL-17F the RC-RC homodimer.
We compared the effects of IL-17A, IL-17F or IL-17A plus IL-17F on bronchial epithelia by monitoring transepithelial ion transport with short circuit current recordings. While both cytokines increase CFTR function, only IL-17A upregulates ENaC activity. We included also brodalumab, a neutralizing antibody against IL-17RA. Brodalumab blocked the upregulation of ENaC and CFTR generated by IL-17A, but did not prevent the upregulation of CFTR caused by IL-17F.
Measurement of apical viscosity by fluorescence recovery after photobleaching (FRAP), revealed that IL-17A promotes a hyperviscous airway surface, whereas IL-17F favors a more fluid environment.
To mimic the CF inflammatory status, we carried out short-circuit current recordings on F508del epithelia treated with ETI, in the presence of IL-17 cytokines and brodalumab. The correcting effect of ETI was markedly amplified by IL-17A, IL-17F, or IL-17A/IL-17F. Importantly, the ability of IL-17F to improve F508del-CFTR rescue was maintained in the presence of brodalumab. These findings support the targeting of IL-17A signaling while preserving the CFTR rescue by IL-17F, suggesting a potential therapeutic role of brodalumab. However, possible side effects of IL17RA inhibition should be considered.

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Epigenome-wide association study and functional analyses reveal regulatory loci linked to lung function and diabetes in cystic fibrosis

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Although cystic fibrosis (CF) is primarily driven by genetic defects, the genotype (*CFTR* variants and modifier loci) does not fully explain the marked clinical heterogeneity observed among patients, especially regarding lung disease. To explore the contribution of determinants to this clinical diversity, we generated genome-wide DNA methylation profiles from blood samples of the MethylCF cohort by hybridization on the Methylation Epic Beadchip (Illumina). Through an epigenome-wide association study (EWAS), we identified differentially methylated CpG sites associated with the disease, lung function (FEV1pp), BMI and cystic-fibrosis–related diabetes. *In silico* analyses (Gene Ontology, Protein-protein Networks and Transcription Factors Binding Sites distribution) pointed to the JAK/STAT and interferon pathways as being affected by methylation changes in blood samples from patients with CF. A significant correlation between DNA methylation levels and clinical traits was replicated for selected CpG sites using pyrosequencing (Pyromark, Qiagen). To address the mechanism that links methylation changes to clinical traits, we examined seven genomic regions containing ten top differentially methylated CpG sites by functional analyses. Using luciferase reporter assays, we brought evidence that four differentially methylated regions in CF (*LYN*, *RGS1*, *SOCS3* and *FADD*) have a gene regulatory function (enhancer, promoter or suppressor) in airways and endothelial cells. Also, in a model of acute inflammation (LPS-treated human monocytes), histone acetylation (H3K27Ac) and transcription levels of the associated genes (*SOCS3*, *LYN*, *SBNO2*, *RGS1*) were increased at four genomic regions, whereas DNA methylation at the selected CpG sites remained stable. Finally, through the survey of the EPIGEN MeQTL (methylation quantitative trait locus) database, we found that five CpG sites were associated with a *cis*-MeQTL. Notably, methylation levels in a CpG site within the *RGS1* gene correlated with cystic fibrosis–related diabetes and was associated with a polymorphic nucleotide variant (rs2816313) that explained 30% of methylation variability. Overall, we conclude that dynamic changes of DNA methylation in cystic fibrosis result from a combination of inherited/genetic factors (i.e. nucleotide variants) and non-inherited/environmental factors, namely chronic inflammation. These findings corroborate and strengthen the results that we previously obtained in nasal epithelial cell and spontaneous sputum samples, while providing additional understanding of the phenotypic variability shown by patients with CF.

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Hypoxia and inflammation drive distinct signalling pathways in human airway epithelia

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Background: Intraluminal mucus plugging associated with cystic fibrosis (CF) and other muco-obstructive lung diseases (MOLDs) renders the airway epithelium vulnerable to localised hypoxia. Adaptation to lowering oxygen tensions is mediated by hypoxia-inducible factors (HIF), particularly the isoforms HIF1A and EPAS1. Hypoxic signatures are present in the airway epithelium of tissue derived from individuals with CF. The hypoxic response induces upregulation of mucin transcription (*MUC5B*) and secretion, along with functional increases in sodium absorption (e.g., γENaC) in airway epithelia. Both of these characteristics are predicted to contribute to mucus hyperconcentration. Inflammation is a persistent contributor to CF lung disease pathology and is well substantiated *in vivo*. Despite the delineation of hypoxia-inflammation cross-talk in other systems, their interactions have not been elucidated in human airway epithelial cells. We sought to investigate these interactions and the regulatory roles of HIF in a human bronchial epithelial (HBE) cell culture model.

Materials and Methods: Differentiated HBE cultures were exposed to chronic hypoxia (1% oxygen) either alone or in combination with supernatants of mucopurulent materials (SMM) derived from excised CF lungs. Two combined exposure protocols were modelled: (1) obstruction-induced hypoxia preceding inflammation or (2) repeated SMM treatment mimicking inflammation prior to the occurrence of hypoxia. CRISPR-Cas9–mediated knockdowns of *HIF1A* and *EPAS1* were used to evaluate their roles in our model. Bulk RNA-sequencing was performed for transcriptional profiling. Ussing chamber experiments investigated the effect of an EPAS1 inhibitor (Belzutifan) on hypoxia-induced changes in sodium absorption.

Results: Transcriptional profiling indicated that hypoxia and SMM exposure orchestrated broadly distinct gene-regulatory responses. A minimal overlap pattern was observed across protocols, while repeated SMM treatment produced a more robust overall response. Co-regulation was evident in the case of a small group of key genes e.g., *PTGS2, CXCL8* and *MUC5B*. HIF1A and EPAS1 were non-redundant in their regulatory roles. In hypoxic HBEs, *HIF1A* knockdown cultures harboured an inflammatory signature with an enrichment of chemokine and extracellular matrix remodelling pathways, and downregulated glycolytic programmes. *EPAS1* knockdown attenuated the induction of *SCNN1G* and *MMP7* while not perturbing glycolysis or upregulating inflammatory pathways. Treatment of HBEs with Belzutifan effectively blunted hypoxia-induced upregulation of sodium absorption.

Conclusion: Hypoxia and inflammatory stimuli govern different transcriptional responses in airway epithelia. Key genes are coordinately regulated and the order of stimulus exposure did not increase the number of shared differentially expressed genes. As both hypoxia and inflammatory signalling are documented *in vivo*, each factor should be considered when designing novel therapeutic strategies. Cellular response to hypoxia is a conserved pathway and EMA- and FDA-approved therapeutics exist. Our studies indicate Belzutifan may effectively blunt hypoxia-induced increases in sodium absorption and suggest that EPAS1 may constitute a new therapeutic target for MOLDs.

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Melanocortins: a pro-resolving approach to control cystic fibrosis airway inflammation

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Background: Cystic fibrosis (CF) lung inflammation remains a main clinical issue, even in the era of highly effective CFTR modulators. It is now established that the resolution of inflammation is actively orchestrated by endogenous mediators, which are being exploited to develop innovative anti-inflammatory therapeutics. Melanocortins are peptide hormones that activate five subclasses of G protein-coupled receptors (MCR1-5). They inhibit NF-κB activation, suppress the release of pro-inflammatory cytokines, regulate leukocyte chemotaxis and promote macrophage efferocytosis. Synthetic melanocortin derivates, like the MCR1 selective agonist BMS-470539 (BMS), display anti-inflammatory and pro-resolving activities, Here, BMS was tested on human CF preclinical systems.

Objective: Main objectives of this work were to:

1. Build a CF human airway preclinical system, utilizing the organ-on-a-chip technology.
2. Assess the impact of BMS on key mechanisms of CF airway inflammation.

Methods: BMS (0.1 — 10 mM) was delivered to a full CF airway-on-a-chip model, constructed by culturing in the upper channel of a Emulate device human primary bronchial CF epithelial cells (HBE), differentiated at air liquid interface (ALI), and in the lower channel human lung vascular CF endothelial cells (HEC). Chips were perfused with PMN, freshly isolated from people with CF (pwCF). In parallel experiments, BMS was added to HBE differentiated in transwells or to HEC monolayers. Experiments were conducted under sterile conditions or in the presence of *Pseudomonas aeruginosa* (PAO1 strain).
Endothelial function was assessed by impedance measurements using the xCELLigence® RTCA apparatus; trans epithelial electric resistance (TEER) was monitored with a voltmeter (EVOM™ Manual); mucus production was quantitated by Alcian Blue staining and MUC5A fluorescence; PMN adhesion and migration were evaluated by confocal microscopy.

Results: BMS (1 and 5 mM) significantly enhanced CF-HEC adhesion, proliferation and barrier integrity ($p < 0.01$). It also reduced CF-PMN adhesion to CF- HEC, both under static conditions ($p < 0.005$) and under flow in PAO1-infected CF airway-on-a-chip ($p < 0.03$). In HBE grown on transwell, BMS increased TEER ($p < 0.0005$), and reduced mucin production both under sterile conditions and PAO1 infection ($p < 0.005$). A significant effect on mucin production was observed in non-infected airway-on-a-chip, ($p < 0.004$). Finally, BMS reduced PMN migration, more effectively when delivered through the airway channel ($p < 0.003$).

Conclusions: These results uncover a significant impact of the small molecule BMS-470539 on key elements of CF lung inflammation, thus opening new opportunities for melanocortin-based pro-resolving approaches in CF. The construction of a full CF airway on-a-chip containing CF-HEC represents a significant advancement for the development of a human preclinical device for drug discovery and personalized medicine.

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Loss of Cftr function in pancreatic stellate cells induces cell activation, fibrogenesis, oxidative stress and lysosomal abnormalities

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Background: Cystic Fibrosis pancreatic pathology is characterized by tissue fibrosis, atrophy and inflammatory cell infiltration. Most research in cystic fibrosis has been focused on dilucidating epithelial cell abnormalities, while investigation on extracellular matrix (fibrosis) producing cells has been neglected. Pancreatic stellate cells (PSC) are the main cell type responsible for fibrosis deposicion in the pancreas. We have shown Cftr is expressed and regulated in PSCs, and reduced CFTR expression in these cells is linked to a proliferative phenotype.

Methods: PSCs were isolated and cultured from C57BL/6 wild-type or Cftr-KO mice. RNA was obtained from PSCs at 75% confluence and used in qRT-PCR and RNAseq experiments. Normalized counts from RNAseq results were examined by Enrichr and GSEA to obtain enrichment pathways scores. Proliferation was assessed using PrestoBlue, migration using Ibidi wound-healing inserts, and invasiveness using Transwell invasion chambers. Apoptotic response to cycloheximide and Tnfa was examined using the Cell Death ELISA kit. Superoxide was measured using DHE. Lysosomal cell distribution, morphology and acidity was assessed in live cells by means of Leica Thunder Imager 3 D microscopy using Lysotracker Red DND-99 and pHLys Red. Lysosomal function was examined using cathepsin D fluorescent probes.

Results: PSCs devoid of Cftr showed increased expression of smooth muscle actin, muc1 and extracellular matrix proteins, both fibrillar (collagens, laminin, fibronectin) and matricellular (lumican, sparc, periostin), as compared to wild-type PSCs. In transcripts from Cftr-KO PSCs, RNAseq analysis disclosed a significant enrichment score in the Epithelial Mesenchymal Transition (EMT) pathway, and marked dysregulation of E2F targets, Inflammatory and INF responses, ROS, complement and G2M checkpoint pathways, and KRAS, TNFa, IL6, IL2 and Hedgehog signaling. Functional studies in Cftr-KO PSCs revealed enhanced proliferation, migratory and matrix-invasive capabilities, as well as increased oxidative stress, resistance to apoptosis and distorted autophagic flux, all and all indicating the adoption of an exalted activated phenotype. In addition, Cftr-KO PSCs contained lysosomes that were smaller in size (both area and perimeter) showing a predominant perinuclear location of acidic lysosomes and overall lysosomal dysfunction.

Conclusion: Impaired CFTR function in pancreatic stellate cells plays a causative role in the generation of the characteristic pancreatic pathology of cystic fibrosis.

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Uncovering the immune architecture of end-stage cystic fibrosis lungs through bulk transcriptomics

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Background: Cystic fibrosis (CF) is a CFTR-related monogenic disorder characterized by viscous mucus, impaired clearance, and progressive airway inflammation. Although classically described as a neutrophil-driven disease, eosinophilic and adaptive immune alterations have increasingly been recognized. Few studies directly examine lung tissue or capture the heterogeneity of small airway disease within individual patients.
Objectives: To define the immune landscape of end-stage CF lungs, characterize epithelial and stromal remodeling, and relate these findings to distinct forms of small airway pathology.
Methods: Frozen explanted lungs from 12 patients with end-stage CF and 8 donor controls were systematically sampled using CT-guided core extraction. Four samples per lung (n = 80) were assessed for tissue structure by microCT and 3D morphometry. Afterwards, the same samples underwent RNA extraction and bulk transcriptomic profiling (Ion AmpliSeq). Immune and stromal cell enrichment was inferred using xCell in R. Findings were validated by quantitative immunohistochemistry.
Results: CF lung tissue showed enrichment of adaptive immune populations alongside epithelial stress, fibroblast activation, and reduced endothelial integrity. Principal component analysis with unsupervised clustering identified three molecular clusters. The first cluster showed preserved endothelial and epithelial features with only mild immune activation, yet demonstrated early upregulation of fibroblast-activation genes. Morphometrically, this cluster corresponded to relatively intact terminal bronchioles and normal tissue density. The second cluster was characterized by pronounced epithelial remodeling together with moderate innate immune involvement. This cluster aligned with increased tissue density and loss of terminal bronchioles. The third cluster demonstrated strong adaptive immune activation, with enrichment of B cells, eosinophils, Th2 signatures, and expression of inflammatory and fibrotic mediators. These features corresponded to high tissue density and extensive terminal bronchiole loss.
Conclusion: End-stage CF lungs exhibit distinct immune and molecular phenotypes rather than a uniform neutrophil-dominant pattern. Different cellular endotypes correlate with morphometric differences seen on microCT. The coexistence of fibroblast-activated, epithelial-driven, and inflammatory–fibrotic endotypes within individual patients indicates that airway-based sampling does not fully reflect underlying tissue pathology. Tissue-level profiling may therefore be essential to guide future targeted anti-inflammatory and anti-fibrotic therapies in CF.

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Stabilization of TTP mRNA as a strategy for inflammation control in CF

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Objectives: The excessive inflammatory response in cystic fibrosis (CF) plays a major role in lung damage. In this inflammatory context, some RNA-binding proteins (RNA-BPs) display deregulated expression. RNA-BPs act by binding to the 3'UTR of mRNAs, inducing their degradation or stabilization. Tristetraprolin (TTP) is an anti-inflammatory RNA-BP that we recently reported to be down-regulated in CF cultures. When non-phosphorylated, TTP destabilizes mRNAs of pro-inflammatory proteins such as IL-6, IL-8, and TNFα, thereby contributing to the resolution of inflammation. We aim to develop strategies to increase TTP levels at both post-transcriptional and post-translational levels, in order to modulate the inflammatory response.

Materials and Methods: TTP gene expression have been assessed by quantitative PCR and TTP protein level assessed by Western Blotting in CF and THP-1 cells. Cytokines levels have been assessed by ELISA dosage and quantitative PCR.

Results: Overexpression of TTP by CRISPRa or expression vectors induced an increase in TTP mRNA and a reduction in pro-inflammatory cytokine mRNA levels, suggesting that elevating TTP mRNA levels promotes inflammation resolution in bronchial cells. As an alternative approach to CRISPRa, we designed chemically modified oligonucleotides (TSB, Target Site Blockers) to increase TTP levels. These oligonucleotides prevent miRNA and RNA-BP recruitment on the 3'UTR of TTP mRNA. Introduction of chemically modified TSBs, encapsulated in cell-penetrating peptides, resulted in a significant increase in TTP mRNA and protein levels in CF cells and macrophages. In an inflammatory context, stabilization of TTP mRNA led to a marked decrease in pro-inflammatory mRNAs and cytokine secretion in CF cells and macrophages. In addition, we are testing another strategy aimed at limiting TTP protein degradation by modulating its phosphorylation state through the use of decoy peptides (mimic TTP and carry phosphorylation motifs). Preliminary results show that introducing these peptides in bronchial cells increase TTP protein levels.

Conclusion: Targeting cis-regulatory motifs on the TTP 3'UTR stabilized both mRNA and protein levels, which was associated with a reduction in pro-inflammatory cytokines. In addition, decoy peptides—an alternative strategy designed to limit protein degradation—were shown to increase TTP protein levels. Taken together, these data confirm that enhancing TTP quantity represents a promising approach for inflammation resolution in cystic fibrosis.

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Neutrophil transmigration across cystic fibrosis bronchial epithelia is modulated by hyperglycemia

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Background: Lifelong, massive migration of neutrophils into the cystic fibrosis (CF) airway in response to chronic infection results in inflammation, which is worsened in CF-related diabetes (CFRD). We hypothesized that during transmigration to the airways, neutrophils interact with bronchial epithelial cells leading damage of the airway monolayer in a variety of ways.
Methods: We utilized our established bronchial epithelial neutrophil transmigration model based upon 16HBE and primary airway epithelial cells expressing WT-CFTR or F508del-CFTR (16HBE-WT, 16HBE-CF, NhBE, and CFhBE). We tested the possible impacts of neutrophil transmigration on the bronchial epithelial monolayer with confocal microscopy, ELISA for neutrophil-based enzymes, bulk RNA sequencing, and immunoblotting.
Results: We found that Matrix Metallopeptidase 9 (MMP9), Myeloperoxidase (MPO), and Neutrophil elastase (NE) were significantly higher in the post-transmigration media compared to control groups without neutrophil transmigration, in NhBE and CFhBE monolayers and was dependent upon glycemic state. In addition, bulk RNA sequencing of NhBE and CFhBE monolayers with and without neutrophil transmigration demonstrated many differentially expressed genes, including genes for gap junctions, tight junctions, glucose transporters, oxidative stress, matrix metallopeptidase, and more, were significantly altered in the groups conditioned to high glucose and with neutrophil transmigration. Interestingly, we also observed that CellTracker dye loaded into neutrophil cytoplasm was frequently exchanged into adjacent bronchial epithelial cells during transmigration, indicating functional interactions between neutrophils and epithelial cells. This phenomenon exhibited a time-dependent increase in frequency.
Conclusion: These data show that neutrophils functionally interact with bronchial epithelial cells during transmigration.

Keywords: Bronchial epithelial cells; neutrophils; transmigration; CFRD; bulk RNAseq

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Microbial crosstalk: influence of *Pseudomonas aeruginosa* on *Mycobacterium abscessus* virulence and antibiotic tolerance

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Background: *Mycobacterium abscessus* (MABSC) is a multidrug-resistant pathogen of increasing clinical relevance in patients with cystic fibrosis (CF). Within the CF airway, MABSC undergoes adaptive evolution associated with enhanced virulence and antibiotic tolerance; however, the selective pressures driving these changes remain largely unknown. The CF lung is a polymicrobial niche in which *Pseudomonas aeruginosa* (Pa) is the dominant co-colonizing organism.

Objectives: To define MABSC–Pa interactions and determine their impact on MABSC virulence and antibiotic response.

Methods: A549 epithelial cells infected with MABSC alone or co-infected with Pa were lysed to quantify intracellular MABSC burden. Virulence was assessed using *Galleria mellonella* survival assays. Antibiotic tolerance was evaluated by time-kill assays in nutrient-rich, nutrient-depleted, and Pa-conditioned media.

Results: Co-exposure of A549 cells to Pa and MABSC significantly increased intracellular MABSC burden compared with MABSC alone ($p<0.0001$). Larvae infected with Pa-conditioned MABSC exhibited reduced survival relative to those infected with non-conditioned MABSC, indicating enhanced virulence. MABSC cultured in Pa-conditioned media demonstrated accelerated killing upon cefoxitin exposure ($p<0.01$) despite preserved exponential growth. This effect was not observed under nutrient-depleted conditions, suggesting potentiation of cefoxitin activity by a Pa-secreted factor. In contrast, Pa-conditioned media did not alter MABSC tolerance to ciprofloxacin or amikacin.

Conclusions: Pa promotes MABSC intracellular infection and virulence, while Pa-derived secreted factors selectively reduce MABSC tolerance to cefoxitin. These interspecies interactions likely shape MABSC pathogenicity and antibiotic response within the CF airway and warrant consideration in the evaluation of therapeutic approaches for MABSC–Pa co-infections.

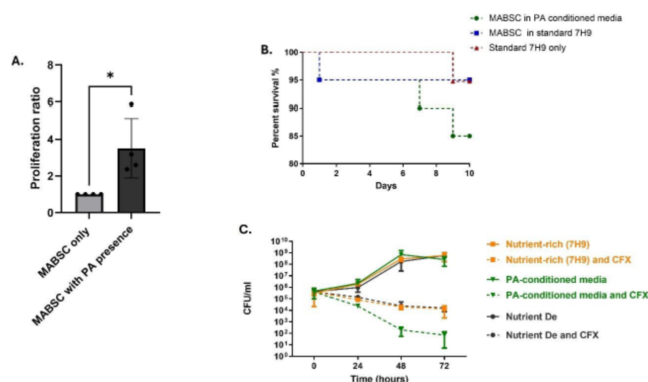

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Fig. 1. Co-pathogen interactions between *Pseudomonas aeruginosa* (PA) and *Mycobacterium abscessus* (Mab). (A) Intracellular infection of A549 alveolar epithelial cells infected with Mab alone or co-incubated with PA. **(B)** Survival of *Galleria mellonella larvae* infected with MABSC grown in standard 7H9 or Pa-conditioned media. **(C)** Cefoxitin time-kill assays of MABSC cultured in nutrient-rich (7H9), nutrient-depleted, or PA-conditioned media. MABSC – *Mycobacterium abscessus*, PA – *Pseudomonas aeruginosa*, CFX – Cefoxitin, Nutrient De- nutrient depleted media

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Metabolic regulation of pulmonary infection trajectory in cystic fibrosis

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Background: Bacterial infection profoundly accelerates pulmonary decline in people with cystic fibrosis (pwCF). *S. aureus* (SA) typically predominates during early childhood, whereas *P. aeruginosa* (PA) emerges later and further entrenches the maladaptive inflammatory environment of the CF airway. Yet the mechanisms underlying this characteristic infection trajectory remain poorly defined. Here, we investigated how the airway metabolic landscape shaped by CFTR mutations, together with the adaptive capacity of CF pathogens, contributes to the progression from SA to PA co-infection, with a particular focus on sulfur-centered metabolic networks.

Aims: This study aimed to determine how CFTR-driven metabolic alterations orchestrate infection trajectories through shared host–pathogen metabolic adaptations.

Methods: Airway metabolic alterations associated with CFTR mutations were examined by LC-MS metabolomics of sputum and airway cell supernatants from CF and non-CF donors. To identify adaptive mutations, we performed whole-genome sequencing (WGS) on SA isolates from CF (n=560) respiratory samples. Functional studies used the JE2 WT strain and its Tn::cysM transposon mutant, which is unable to convert L-homocysteine into L-cystathionine, L-cysteine, and taurine. Bacterial growth was assessed in LB and artificial sputum medium (ASM) supplemented or not with sulfur-rich metabolites (L-methionine, L-homocysteine, L-cystathionine, L-cysteine, taurine). Infection outcomes — including bacterial burden (CFU enumeration) and secretion of CF-relevant cytokines (ELISA) — were evaluated in non-CF and CF airway epithelial (F508del/W1282X) and differentiated myeloid cells (F508del/F508del) using gentamicin protection assays. *In vivo* relevance was assessed using a mouse model of respiratory infection.

Results: CF sputum and supernatants from CF airway epithelial cells were enriched in sulfur-containing metabolites, including L-methionine, N-acetyl- methionine, L-cystathionine, taurine, and S-cysteinosuccinate. WGS revealed that ~78% of SA isolates from pwCF harbor inactivating mutations in *cysM*, a gene essential for the generation of cysteine through the conversion of L-homocysteine into L-cystathionine. Functional studies in LB demonstrated that *cysM* inactivation confers resistance to the stress imposed by sulfur metabolites like cysteine. ASM provided similar protection to *cysM*-intact strains, supporting an adaptive convergence between bacterial genetics and the CF metabolic milieu. In human CF epithelial cells, *cysM* inactivation enhanced IL-8 secretion.

Although this mutation did not increase SA persistence in neutrophils, during co-infection with PA it markedly promoted PA survival — an effect reproduced *in vivo*.

Conclusion: These findings reveal that the sulfur-rich airway environment generated by CFTR dysfunction imposes selective metabolic pressure favoring *cysM*-deficient SA strains. These organisms are more resilient to the CF metabolic milieu, elicit heightened epithelial inflammation, and — critically — facilitate PA persistence. Together, our data provide a metabolic explanation for the characteristic polymicrobial infection trajectory in CF and identify sulfur metabolism as a key driver of pathogen evolution and community dynamics in the CF airway.

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Long-standing adaptation and regulatory plasticity in Pseudomonas aeruginosa during CFTR restoration

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Elexacaftor/tezacaftor/ivacaftor (ETI), a highly effective CFTR modulator combination, is transforming cystic fibrosis (CF) care, driving profound improvements in lung function, exacerbation burden, and quality of life. Yet, despite these gains, chronic *Pseudomonas aeruginosa* infection often persists in people with CF (pwCF) beginning ETI, revealing resilience to the improved airway environment. Additionally, therapeutic benefit varies widely among individuals, prompting renewed investigation into microbial contributions to treatment response.
To investigate pathogen adaptation under CFTR restoration, we implemented a longitudinal framework integrating phenotypic assays, whole genome (WGS) and transcriptome (RNAseq) sequencing, and clinical outcome analysis. Fourteen chronically infected people with CF initiating ETI contributed sputum samples before and during therapy. A total of 54 *P. aeruginosa* isolates were collected, and 35 isolates from 11 participants underwent short- and long-read WGS to construct personalized reference genomes and track within-host evolution over time. Phenotypic traits associated with chronic infection, including motility, biofilm formation, pyocyanin and protease production, mucoidy, growth rate, and antibiotic susceptibility, were systematically evaluated. Six isolates were additionally exposed *in vitro* to ETI to identify transcriptional responses directly induced by modulators, assessed by RNAseq. Clinical outcomes were analyzed using 12-month windows: the year before ETI initiation and the year preceding the final post-ETI sampling for each participant. Relationships between pathogen and pwCF features were explored using Factorial Analysis of Mixed Data.
Classical chronic phenotypes persisted across isolates, including mucoidy, multidrug resistance, reduced motility, and lasR-associated traits. Genomic diversification continued, but with minimal overlap of mutated genes across individuals, consistent with individualized evolutionary trajectories.
Transcriptomic profiling revealed strain-specific yet pathway-convergent changes involving metabolism, virulence, stress responses, and β-lactam resistance, suggesting shared selective pressures despite divergent genetic routes. Several of these transcriptional signatures also appeared after *in vitro* exposure to ETI, supporting the possibility that CFTR modulators exert direct effects on bacterial regulatory pathways. Phenotypic assays did not reveal corresponding changes under the tested conditions, suggesting that early and reversible regulatory adjustments may require more prolonged exposure to translate into detectable traits.
Clinically, participants experienced significant improvement, including a median 15.5% increase in FEV_1 percent predicted and a reduction in exacerbations, hospitalizations, and intravenous antibiotic use. Individuals with the greatest benefit tended to harbor isolates with loss or absence of mucoidy and the LasR-associated phenotype, persistent or emerging hypermutability, and reduced pyocyanin production, suggesting that pathogen features may relate to treatment efficacy. These findings show that while ETI markedly improves the airway environment and clinical outcomes, *P. aeruginosa* persists through deeply rooted adaptations and responds primarily through flexible regulatory rewiring. Monitoring pathogen evolution during ETI therapy may support precision management of chronic infection in the evolving CF landscape.
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A virtual screen-based approach to identify bacterial cell division inhibitors against cystic fibrosis pathogens

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Lower airways bacterial infections are one of the main causes of morbidity and mortality in people with cystic fibrosis (pwCF). Multidrug-resistant (MDR) *Staphylococcus aureus* and *Pseudomonas aeruginosa* are the most frequently isolated pathogens. They can persist and adapt to hostile airway niches, and they are equipped with many virulence factors.
In the search for new compounds able to tackle MDR strains, attention was focused on bacterial cell division. Indeed, FtsZ inhibitors represent a valuable option, so the divisome proteins FtsZ and FtsA/FtsN were targeted using Computer-Aided Drug Design (CADD) methods.
On one hand, by a structure based virtual screening approach it was selected derivative C11. The compound showed in vitro to act through FtsZ polymerization inhibition. The *in vivo* experiments clarify a bacteriostatic activity with an MIC of 2 µg/ml against *S. aureus* and CF MRSA clinical isolates, using also the synthetic cystic fibrosis sputum medium (SCFM). The combination of C11 with β-lactams restored methicillin susceptibility to MRSA strains. Moreover the compound can interfere with *S. aureus* biofilms in the *ex vivo* pig lung model. C11 increased survival in a *Galleria mellonella* infection model, and it is now tested in mice.
On the other hand, a similar research approach was implemented to study a new target: the *P. aeruginosa* FtsZ-FtsA complex. This complex plays a vital role for bacteria, and numerous proof-of-concept studies show that impairing its formation has an antibacterial effect.
Firstly, the FtsA/FtsN complex of *Vibrio maritimus* was inspected by molecular dynamics to identify interactions that stabilize the proteins complex. Then, the FtsN binding pocket was used to dock a library of commercially available compounds. The obtained binding conformations were sorted according to a pharmacophore model derived from information gained from molecular dynamics simulations. The compounds with the best superimposition to the pharmacophore model were retained and further visually analysed. Nine structurally unrelated molecules were selected for biological evaluation. To rule out their interaction with FtsA enzymatic activity, it was demonstrated that none of the compounds interferes with FtsA ATPase activity. Then, a pull-down assay was set up showing that four compounds can block the interaction between the two proteins. To investigate their mechanism of action against *P. aeruginosa* cells, a strain impaired in the lipoprotein-processing pathway was necessary since the compounds did not retain any activity against bacterial growth. Further optimisation of these molecules will be necessary to improve the efficacy against *P. aeruginosa*.
Taken together, our results show that virtual screening is a rapid and effective approach, and it enabled us to identify two new active molecules that are promising for future development.

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Hypochlorite regulates an epoxide-hydrolase virulence circuit in *Pseudomonas aeruginosa*

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Background: *Pseudomonas aeruginosa* secretes the epoxide hydrolase Cif into the airway of people with cystic fibrosis (pwCF). Acting as a virulence factor, Cif reduces the cell-surface abundance of CFTR and also triggers hyperinflammation, leading to worse outcomes for pwCF. Cif helps *P. aeruginosa* detoxify environmental chemicals, and its expression can be triggered by the presence of reactive epoxides. However, the regulation of *cif* expression in the context of pwCF airway is unknown.
Methods: We performed structural studies on CifR, a TetR family transcriptional repressor, which regulates *cif* expression. Using purified CifR protein and synthetic operator DNA derived from the cif promoter, we conducted a compound library screen to identify chemicals capable of disrupting the CifR–DNA complex. Various biochemical approaches were applied to reveal the mechanism of regulation by small molecule compounds.
Results: We determined the molecular structures of CifR alone and in complex with operator DNA, resolved in a single crystal lattice. Significant conformational changes between these two structures revealed how CifR regulates the expression of virulence gene *cif*. We also determined that cysteine residue Cys107, located at the center of the ligand binding domain as indicated by the structure, is critical for epoxide sensing and DNA release.
Mechanistically, epoxides can covalently modify Cys107. In addition to epoxides, we showed that sodium hypochlorite can release CifR from operator DNA, leading to *cif* overexpression. Similar to regulation by epoxides, hypochlorite-sensing is also dependent on Cys107. Mass spectrometry data suggests that Cys107 undergoes oxidation upon exposure to hypochlorite. These results together suggest that modifications on Cys107 may prompt the CifR conformational change, triggering its dissociation from the operator DNA at the *cif* promoter.
Conclusions: This study offers new insights into the stereochemical regulation of a bacterial epoxide-hydrolase virulence circuit relevant in the context of chronic CF airway infections that can persist in spite of modulator therapies. Other than epoxides, we found that CifR can sense hypochlorous acid, which can be produced by neutrophils in response to bacterial infections. These results suggest that the operon has multiple sensing mechanisms involving signals derived from its colonizing host. More broadly, *cif* overexpression has been shown to sabotage pro-resolution immune signals, suggesting that this regulatory pathway has the capacity to generate a feed-forward signal leading to hyperinflammation.

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Pseudomonas aeruginosa induced GM-CSF production by airway epithelial cells could help defend against Mycobacterium abscessus

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Rationale: People with cystic fibrosis (pwCF) are at high risk for developing nontuberculous mycobacteria pulmonary disease (NTM-PD), which is difficult to treat and increases morbidity and mortality. Yet only a fraction of pwCF develop NTM-PD (10-15%). To better understand heterogeneity in NTM-PD susceptibility, the role of granulocyte-macrophage colony stimulating factor (GM-CSF) in host resistance to NTM-PD caused by *Mycobacterium abscessus* (*Mabsc*) was investigated. It is known that GM-CSF is essential for murine defense against *Mabsc* acute pneumonia. Therapeutic nebulized GM-CSF is being tested for recalcitrant NTM-PD in pwCF and non-CF bronchiectasis, with some successes. GM-CSF is constitutively produced by alveolar pneumocytes; however, epithelia in the airways (where bronchiectasis occurs) rarely transcribe *CSF2* (GM-CSF), but *Pseudomonas aeruginosa* (*Pa*) can induce GM-CSF production by epithelial cell lines in vitro. Whether *Pa* can stimulate GM-CSF production by primary airway epithelia in vitro or in vivo has not been investigated. Moreover, which immune cells respond to GM-CSF to protect against *Mabsc* remain unknown. We hypothesized that specific *Pa* isolates can stimulate airway epithelial GM-CSF production, and that focal airway GM-CSF programs macrophages for effective killing of *Mabsc.*

Methods: Human monocyte-derived macrophages (MDMs) from healthy donors were matured with or without GM-CSF, infected with *Mabsc*, and colony forming units were determined after 96 hours. GM-CSF reporter mice (genetically engineered to produce a bicistronic mRNA encoding *Csf2* and TdTomato under control of the *Csf2* promoter) were inoculated oropharyngeally with 100-200 micron diameter agar beads (which reliably lodge in small airways) containing heat killed *Pa*, live *Mabsc*, or no infection. Primary human bronchial epithelia differentiated to model small airways as air-liquid interface (ALI) cultures were exposed to *Pa* or Mabsc*;* RNA abundance and secreted cytokines were quantitated.

Results: GM-CSF matured MDMs showed enhanced ability to kill *Mabsc* compared to those matured in M-CSF, which demonstrated minimal *Mabsc* killing ($p<0.001$). Immunofluorescence microscopy of GM-CSF reporter mice lungs revealed that heat killed *Pa* beads but not sterile beads nor *Mabsc* beads induced GM-CSF production by airways epithelial cells. ALI cultures exposed to *Pa* demonstrated increased *Csf2* mRNA ($p<0.0073$) and apical GM-CSF protein expression ($p<0.019$) at 24 hours compared to unstimulated cells and cells exposed to *Mabsc.*

Conclusions: *Pa*-induced GM-CSF production by airway epithelia may create focal environments where recruited MDMs become programmed to develop anti-*Mabsc* phenotypes. Ongoing studies aim to (a) create murine co-infection models to test whether *Pa* co-infection decreases *Mabsc* bacterial burden in mice inoculated with *Mabsc* agar beads, and (b) determine if in-host evolution drives development of *Pa* isolates that lose ability to induce airway GM-CSF by testing early vs late clinical *Pa* isolate pairs in vitro and in vivo.

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ETI modulates airway inflammation and reduces Pseudomonas aeruginosa burden in pediatric cystic fibrosis

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Background: In cystic fibrosis (CF), inflammation drives disease progression. This heightened inflammatory state results from several converging mechanisms, including direct pro-inflammatory effects of CFTR mutations, epithelial hypoxia arising from mucus stasis, and pathogen-induced inflammation. *Pseudomonas aeruginosa* (*Pa*), the most common bacterial pathogen in CF, further amplifies airway damage by triggering neutrophil recruitment and protease release, notably neutrophil elastase (NE). While the anti-inflammatory properties of triple combination therapy (ETI) are increasingly recognised, its impact on host immune defences and bacterial burden remains insufficiently characterised.

Aims: This study aimed to evaluate how treatment controls *Pa* burden and reshapes innate immune responses in children with CF.

Methods: Induced sputum samples were collected within the multicenter observational study MODUL-CF (NCT04301856). Longitudinal sputum cultures were analysed at baseline and after 12 months of ETI therapy allowing classification of patients as chronic, intermittent, or negative. To assess the impact of ETI on bacterial burden and host defence, sputum concentrations of antimicrobial peptides (Cathelicidin, SLPI and β-defensins) were measured before and after therapy alongside NE, calprotectin, IL-1β, IL-6, IL-8 and TNF-α, and were compared to 13 healthy controls. To complement these *in vivo* experiments, primary nasal epithelial cells from healthy donors, Phe508del individuals, and patients carrying a premature termination codon (PTC) were cultured at the air-liquid interface (ALI), treated with ETI or vehicle (DMSO) and infected with *Pa*. Bacterial growth was monitored across genotypes with and without treatment.

Experiments were repeated in the presence of a neutralizing anti-SLPI antibody to assess the contribution of this major antimicrobial peptide, and supernatants were collected to quantify inflammatory markers.

Results: Notably, after 12 months of ETI therapy, and in the absence of concurrent antibiotic treatment, a subset of patients cleared *Pa* from their airways. In a separate cohort, sputum concentrations of the antimicrobial peptide SLPI increased significantly after treatment ($p<0.01$), accompanied by reductions in pro-inflammatory markers (NE, calprotectin, IL-1β, IL-6, IL-8, and TNF-α) although levels remained above those observed in healthy controls. Importantly, the pattern observed in ECBC data was recapitulated *ex vivo* in *Pa*-infected primary epithelial cells. Incubation with ETI reduced bacterial growth in cells from CF patients ($p<0.05$), but not in cells from healthy donors ($p=0.73$) or individuals carrying a PTC ($p=0.55$). Notably, the observed reduction was not due to a direct antibiotic effect, suggesting that ETI induces host-mediated mechanisms limiting *Pa* growth. Neutralization of SLPI with a specific antibody reduced the ETI-mediated inhibition of bacterial growth, suggesting a key role for SLPI in controlling *Pa* under therapy.

Conclusion: ETI not only exerts potent anti-inflammatory effects but also enhances host antimicrobial defences, suggesting a dual role in limiting infection and shaping airway innate immunity in children with CF.

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De novo pyrimidine reprogramming shapes cystic fibrosis lung disease

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Introduction: Cystic fibrosis (CF), caused by CFTR mutations, leads to airway dehydration, chronic epithelial injury, and structural remodeling that drive progressive respiratory decline. In this altered environment, *P. aeruginosa* — the most dominant CF pathogen — persists and evolves, most notably through *mucA* mutations that produce the mucoid phenotype resistant even to CFTR modulators. However, the mechanisms shaping this maladaptive host-pathogen landscape remain unclear. Here, we identify the *de novo* pathway of pyrimidine synthesis (DNPPS) as a shared metabolic axis that drives both aberrant CF epithelial cell proliferation and the increased biomass of mucoid *P. aeruginosa*. This glutamine-fueled pathway — well known for supporting hyperproliferative and immune-evasive cells like tumors — emerges as a central dependency in the CF lung. By leveraging therapeutic strategies used in oncology and autoimmunity to curb uncontrolled cell proliferation, we identify actionable DNPPS targets in CF, including DHODH in host cells and *pyrD* in *P. aeruginosa*, each serving as the rate-limiting enzyme controlling pyrimidine flux — dihydroorotate dehydrogenase. Together, these findings redefine CF lung disease as a coordinated host-pathogen pyrimidine rewiring and reveal therapeutic opportunities beyond current antimicrobials and CFTR modulators.

Methods: To define the role of DNPPS in CF epithelial overproliferation, we treated primary bronchial epithelial cells (ΔF508/ΔF508 and WT/WT) and airway cell lines (ΔF508/W1282X and WT/WT) with: 1) DHODH inhibitors (leflunomide, teriflunomide, brequinar, gemcitabine); 2) DHODH-targeting siRNA; and 3) CFTR modulators (ETI). To assess DNPPS in mucoid *P. aeruginosa* biomass production, we used a murine lung infection model inoculated with *mucA22* PAO1, its *mucA22ΔpyrD* isogenic mutant, or WT PAO1; PBS served as control. DHODH inhibition *in vivo* was achieved using leflunomide (25 mg/kg). Pulmonary cytokines and bacterial burdens were quantified by ELISA and plating, respectively. Intracellular and extracellular metabolites, along with ^{13}C-glutamine carbon flux, were analyzed by LC-MS-based metabolomics

Results: Our findings demonstrate that CFTR-mutant airway epithelial cells and mucoid *P. aeruginosa* both channel glutamine into DNPPS to drive hyperproliferation and establish a remodeled, immunosuppressed environment that promotes bacterial persistence. This DNPPS-dependent niche not only supports a host-pathogen consortium resembling hyperplastic pathological tissues but also enables the buildup of immunometabolites — such as itaconate — that further promote *mucA* inactivation in *P. aeruginosa*. Strikingly, disrupting this axis by inhibiting DHODH in host cells or deleting *pyrD* in *P. aeruginosa* limited this maladaptive milieu, revealing clear therapeutic targets beyond conventional approaches.

Conclusion: We demonstrate that *P. aeruginosa* persistence in the remodeling CF lung is fueled by glutamine-driven activation of the DNPPS pathway within the airway epithelium. By defining a direct link between CFTR dysfunction and aberrant pyrimidine metabolism, our work uncovers therapeutic strategies that move beyond CFTR modulators and traditional antibiotics — an especially critical advance for individuals with CFTR variants, including class I mutations, that remain refractory to current treatments.

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Leveraging metabolic signatures induced by CFTR modulator therapy to eradicate lung infection

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Introduction: Chronic lung infections with extracellular pathogens *P. aeruginosa* and *S. aureus* remain major drivers of morbidity in cystic fibrosis (CF). Despite strong immune activation, these organisms persist, withstand repeated antibiotic exposure, and accelerate lung decline. Although CFTR modulators substantially reduce bacterial burden, they rarely achieve complete eradication, leaving patients vulnerable to recurrent flares and ongoing antibiotic resistance. Thus, new strategies are needed to enhance bacterial clearance and preserve lung function in the modulator era. Here, we leveraged CFTR modulator–induced metabolic programs to improve pathogen clearance through antibiotic-dependent and -independent mechanisms. We focused on purine and ketogenic signaling, two interconnected pathways that preserve tissue integrity during inflammation. Using *in vitro* and *in vivo* models, manipulating these pathways potentiates tobramycin or reduces bacterial loads through nutrient-driven mechanisms, particularly via purine synthesis or fatty acid–driven ketogenesis, respectively. These findings outline a metabolic framework to strengthen current therapies and improve eradication of persistent CF airway pathogens.
Methods: To evaluate how CFTR modulators reshape extracellular metabolism, we performed LC-MS metabolomics on supernatants from human phagocytic cells (DF508/DF508 and WT/WT CFHL60) and airway epithelial cells (DF508/W1282X and WT/WT IB31/C38). To test how purine signaling affects antibiotic susceptibility, we: 1) measured *P. aeruginosa* (PAO1) and *S. aureus* (USA300) survival to tobramycin ± adenosine; and 2) assessed tobramycin efficacy in infected animals that cannot generate extracellular adenosine ($Cd73^{-/-}$), using $Cd73^{+/+}$ littermates as controls. To define how ketogenesis influences bacterial clearance — and whether this depends on purine signaling — we: 1) fed mice a fatty acid–rich diet to induce ketogenesis or a carbohydrate-rich control diet; and 2) repeated these experiments in $Cd73^{-/-}$ hosts. Across all *in vivo* studies, we quantified survival, airway inflammation, bacterial burden, and local/systemic ketone levels. CF sputum metabolomics were also performed.
Results: Our findings reveal that CF phagocytic cells, but not epithelial cells, adopted a metabolic state dominated by ketogenesis before and after CFTR modulation. In the human lung, this signature was closely coupled to purine signaling as confirmed by sputum metabolomic profiling. *In vivo*, the ketogenic program was shown to arise downstream of purine activation, driven specifically by CD73-mediated purine metabolites. Strikingly, both metabolic pathways enhanced clearance of *P. aeruginosa* and *S. aureus* from the respiratory tract, independent of inflammatory responses. Whereas the CD73-adenosine axis potentiated tobramycin activity, ketogenic nutrition directly reduced bacterial survival. Parallel *in vitro* experiments corroborated these observations.

Conclusion: Our study identifies a distinct immunometabolic signature induced by CFTR modulator therapy — purine and ketogenic pathways — and clarifies how these changes enhance bacterial clearance in the CF airway. By defining how modulators reinforce antimicrobial capacity, we highlight actionable metabolic strategies that can be harnessed to further amplify these networks, particularly in subjects refractory to CFTR correction.

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Exhaled breath condensate analyses identify specific human protein signatures for detecting individual airway pathogens in people with cystic fibrosis

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Background: People with Cystic Fibrosis (pwCF) suffer from chronic airway infections. While these infections are typically polymicrobial, the presence of bacteria considered “classic” CF pathogens is associated with accelerated lung function decline. Historically, sputum cultures have been the standard for monitoring airway infections. However, CFTR modulators have significantly decreased or eliminated sputum production in most pwCF. While modulators reduce airway bacterial burden, chronic infections persist. This creates a critical diagnostic gap: the inability to monitor airway infections non-invasively in pwCF. Emerging methods such as cell-free microbial DNA detection in blood and volatile organic compound profiling offer alternatives, but each has limitations. Analysis of exhaled breath condensate (EBC) could fill this void.

Methods: EBC samples were collected from 46 pwCF after breathing through an RTube™ (Respiratory Research, Austin, TX, USA) for 10 min, yielding ~2 mL.

One mL EBC was dried and resuspended in lysis buffer following the S-trap micro protocol. Peptides were injected using the nanoElute 2 (Bruker) and separated on a PepSep C18 column (150 um x 250 cm, 1.9 um, Bruker) over a 45 min linear gradient. Samples detected by data-independent acquisition (DIA) against the Bruker Human Spectral Library and a Uniprot human database. Samples were reinjected and detected by data-dependent acquisition (DDA) analysis and searched against a database containing proteomes from six common CF bacteria as well as a human protein database. Data were searched in DIA-NN (DIA) or with MSFragger within Fragpipe (DDA). Data analysis involved LASSO regression, while differential expression analysis of human proteins was conducted using linear modeling with the limma R package.

Results: EBC analysis from 46 pwCF detected 322 - 1,577 proteins (average 670) in each sample. There were 179 proteins common to all EBC collections. Since the bacterial compared to the human protein abundance in EBC is relatively low, we carried out a detailed statistical analysis of the changes in human proteins in each sample to determine bacterial infections from human proteome changes. In EBC from pwCF previously diagnosed with S. aureus, 141 proteins were significantly different from the other samples with the ability to differentiate between MSSA and MRSA. EBC from P. aeruginosa infections showed 217, from S. maltophilia 61, from Achromobacter species 75, and from M. avium 18 significantly different proteins from other samples.

Conclusions: Our results demonstrate the power of quantitative proteomics to distinguish specific bacterial infections based on differentially expressed human proteins. Further prospective analysis is required for validation. In addition, protease activities in these samples may give insights into virulence of the detected pathogens.

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CFTR protein expression in epithelial tissues of a 2XHA-CFTR mouse model

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CFTR detection in native tissues has been hampered by low specificity of available antibodies to detect the channel in the mouse. We designed a strategy to generate a mouse model that allows the detection of a fusion CFTR protein with the haemagglutinin (HA) epitope, to detect CFTR using highly specific anti-HA antibodies.
Using the CRISPR Cas9 technology we inserted 2 HA epitopes in the amino-terminal of the mouse *Cftr* gene and confirmed the gene modification by sequencing. The parental lines were able to transfer the modification to the descendants and no changes were observed in the weight of 2XHA-CFTR animals compared to their wild type and heterozygous siblings, suggesting that the insertion of the HA epitopes doesn't altered CFTR channel function.
Western blot analysis using an anti-HA antibody detected a ~150kD band in parotid and submaxillary salivary glands, jejunum, ileum and colon of heterozygous animals. Using the anti-HA antibody we observed CFTR on the apical membrane of NKCC1+ and NKCC1- epithelial cells of the ducts of the parotid gland. CFTR was also detected in the apical membrane of NKCC1+ cells of the ileum and colon crypts of the mouse intestine. Finally, we observed scattered CFTR+ cells with strong signal on the apical membrane that resemble CFTR-rich cells or pulmonary ionocytes and other epithelial cells with intracellular signal in granules. Currently, we are using anti-FOXI1 and anti-ASCL3 antibodies to corroborate CFTR expression in ionocytes and detecting the fusion protein in other tissues, as the gallbladder, pancreas and the central nervous system. The use of this model will aid detect CFTR during different stages of development and determine changes in expression during induced models of diseases in the mouse.

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Application of a semi-automated image analysis algorithm and respiratory organoids for cystic fibrosis drug screening

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CFTR modulators are the most effective treatment for cystic fibrosis (CF), but they are not suitable for all patients. To support new therapy development, a representative *in vitro* model is essential for drug screening. This study evaluated human induced pluripotent stem cell-derived lung organoids (hiLOs) from F508del patients as a 3D model for assessing CFTR modulator efficacy using the forskolin-induced swelling (FIS) assay.
hiLOs were generated from four donors with CF (*CFTR* F508del/F508del). FIS assessed CFTR channel function recovery after exposure to modulators (VX-770 with correctors VX-809, VX-661, or VX-661/VX-445). Image analysis used a novel semi-automated algorithm based on U-Net convolutional neural network, validated for both primary nasal organoids and hiLOs. This enabled accurate, dye-free quantification from bright-field images (IoU = 0.8856, F1-score = 0.937). The statistical analysis was conducted using GraphPad Prism v.9.1.1.
All four hiLO lines demonstrated a statistically significant increase in area (mean increase of 111%) in response to the triple combination therapy (VX-770/VX-661/VX-445), indicating the restoration of CFTR channel function. In contrast, treatment with double combination modulators, VX-770/VX-809 and VX-770/VX-661, resulted in less effective CFTR channel recovery (mean area increases of 46% and 38%, respectively). Notably, hiLOs derived from healthy donors exhibited a 98% increase in area in response to forskolin. Two were receiving CFTR modulator therapy (Donors 1 and 2). Donor 1 (male, 33 y.o.) had a baseline ppFEV1 of 20.8% and a sweat chloride concentration (SSC) of 82 mmol/L at the start of therapy with VX-770/VX-809. Over one year of treatment, ppFEV1 remained stable at approximately 20%. *In vitro* evaluation of hiLOs derived from this donor's cells showed a minimal or absent response to the modulators (mean area increases of 51%). Donor 2 (female, 26 y.o.) had a baseline ppFEV1 of 75% and an SSC of 111 mmol/L before starting VX-770/VX-809 therapy. After two years of treatment, ppFEV1 declined to 51%, and SSC decreased to 85 mmol/L after six months. Subsequently, the patient was switched to the triple combination therapy. Four months into this treatment, ppFEV1 increased to 88%, and SSC decreased to 56 mmol/L. *In vitro* evaluation of hiLOs derived from this donor's cells demonstrated a similar response dynamic to the CFTR modulators used in the patient's clinical therapy (mean area increases of 40% (VX-770/VX-809) and 95% (VX-770/VX-661/VX-445)).
The integration of the U-Net-based analysis pipeline enabled high-throughput, accurate quantification of FIS without fluorescent dyes, overcoming limitations of manual analysis and reducing potential cytotoxicity. The results of the work demonstrate the potential of hiLOs as a model for assessing CFTR modulator therapy using the FIS assay. These findings suggest that hiPSC-derived lung organoids are a promising platform for CF drug screening.

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Improving reproducibility, robustness and standardization of CFTR function measurements in PDIOs

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Background: CF patient derived intestinal organoids (PDIOs) have been used over 10 years to determine baseline CFTR activity, responsiveness to drugs and to screen for novel therapies. Developments in technology facilitate further improvements in culturing conditions and assay design.

Reliable and scalable expansion of undifferentiated patient-derived intestinal organoids (PDIOs) is essential for robust assays, as CFTR-expressing stem cell presence is key for CFTR functional characterization. The 3D-matrix that encapsulates PDIOs is a crucial ingredient for this. The current golden standard Matrigel is subject to batch-to-batch differences, finding an alternative with more consistency could enhance long term reproducibility of PDIO growth and assay performance. Another defining factor in organoid growth is the medium containing growth factors and nutrients. We are currently characterizing replacements for Noggin conditioned medium (NCM) and Wnt conditioned medium (WCM).

In parallel, we aim to modernize functional CFTR assays. The original FIS assay relies on Calcein Green, which limits imaging duration due to photobleaching. Recently developed stain-free, brightfield-based analysis (OrgaSegment) enables extended imaging and is better suited for high-throughput applications.

Besides FIS, another assay to give an indication of CFTR function in PDIOs is steady-state lumen area (SLA). Although SLA is a useful tool, obtaining the measurements is laborious. We aim to automate SLA by training an artificial intelligence (AI) model to differentiate between PDIOs with a range of CFTR function. A last ongoing aspect of advancing functional CFTR characterizations, is implementation of an automated robotic platform integrating incubation, liquid handling, and imaging, which will further increase throughput and experimental reproducibility compared to manual workflows.

Methods: For each area of focus (growth conditions, assay optimization & automation), we set out to compare PDIO growth and CFTR functional rescue across a range of genotypes.

Results: Pilot experiments with a Matrigel alternative are challenging, yet one alternative holds promise, potentially when combined with media supplemented with additional growth factors. We are currently comparing commercially available growth factors to replace WCM and NCM, with promising pilot-data.

The SLA substitution AI trained model to classify organoids based on their phenotype is being tested with several datasets. Currently, the model is able to distinguish with certainty whether a picture with 20+ PDIOs is from a healthy individual or from someone with severe CF. The next step is whether the model can classify PDIOs with a range of CFTR function. We have used our robot platform for several high-througput screens with satisfactory results.

Conclusion: By optimizing our PDIO culture conditions and our assays and by utilizing the robot platform, we can generate more data that is of high, more consistent quality. These improvements will facilitate the diagnosis of individuals with CF and aid in identifying the most effective treatment for each individual.

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Uncovering how ETI therapy reshapes chronic Pseudomonas infection in primary cystic fibrosis bronchial epithelial cultures

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Cystic Fibrosis (CF) is characterized by mutations in the CF Transmembrane Conductance Regulator (CFTR) gene, which encodes an epithelial ion channel involved primarily in the conduction of chloride and bicarbonate ions. Disrupted CFTR function impairs multiple epithelia-lined organs, with the lungs being most profoundly affected. In fact, the leading cause of fatality in people with CF (pwCF) is lung failure due to chronic and recurrent bacterial infections. Highly effective modulator therapies (HEMTs) that directly target and rescue the CFTR defect demonstrate marked clinical improvements in over 90% of pwCF. However, they do not fully eradicate lung infections and their impact on bacterial growth in airway cells is not completely understood. To explore the reciprocal interactions between HEMT and bacterial infections, we established a bacteria-epithelia co-culture model using *P. aeruginosa* (*Pa*), an opportunistic pathogen highly prevalent in adult CF airways, and CF primary bronchial air-liquid interface (ALI) cell cultures treated with the triple combination HEMT elexacaftor/tezacaftor/ivacaftor (ETI) consisting of two CFTR corrector compounds (ET) and a CFTR poteniator (I). We optimized culture conditions to achieve the delicate balance between an active apical *Pa* infection and maintaining epithelial cell integrity and were able to retrieve metabolically active *Pa* at the end of the infection period without basolateral dissemination. Our novel in vitro model allows a 72-hour infection period, enabling us to study the impact of persistent bacterial infection in primary cells. In *Pa* infected primary human CF bronchial epithelial cells (HBE), we observed significant changes in expression of genes involved in key biological pathways such as hypoxia, immune response, inflammation, and extracellular matrix organization. Since recurrent *Pa* infection is still a major cause of mortality in pwCF post-ETI treatment, we evaluated the impact of ETI on bacterial growth. Consistent with recent reports, we observed a significant decrease in bacterial burden in ETI-treated CF HBE cells. Interestingly, we also observed a reduction in *Pa* growth in the basolateral media from infected, ETI-treated CF HBE cells, suggesting the presence of secreted factors impacting bacterial burden. Further, transcriptomic analysis revealed that ETI treatment impacted genes involved in immune response, inflammation, and metabolism. Our results suggest that in addition to restoration of CFTR function, other downstream changes induced by ETI may play a role in reducing bacterial burden in diseased epithelial cells. By deepening our understanding of how HEMT and bacterial infection influence host-pathogen interactions in the CF lung, this study has the potential to inform the development of more effective therapies, ultimately aiming to improve the quality of life and long-term health outcomes for al pwCF.

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Preclinical model of precision cut lung slice for the evaluation of therapeutic treatments in CF

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Background: Current preclinical models used in cystic fibrosis (CF) research, such as immortalized cell lines, primary cultures of human bronchial or nasal epithelial cells, or organoids derived from people with CF (pw-CF), are valuable for assessing CFTR modulator (CFTRm) efficacy. However, they do not fully recapitulate pulmonary tissue architecture or the multicellular microenvironment, including epithelial, endothelial, and immune compartments. Precision-cut lung slices (PCLS) derived from healthy lung tissue or explanted lungs from pw-CF preserve native epithelial structure and cell diversity, making them a highly promising translational *ex vivo* preclinical model for investigating responses to infectious and inflammatory environments, as well as CFTRm, in living tissues over extended periods.

Objective: To develop and validate an optimized PCLS protocol that maintains viable human pulmonary tissue over prolonged cultures, preserves key structural and functional properties, and enables reproductive exposure to infectious or inflammatory stimuli, as well as assessment of responses to CFTRm.

Methods: We optimized sampling procedures, agarose infusion, slicing using a Compresstome, and cultures conditions to minimize tissue injury, maximize the number of serial slices obtained, and support long-term viability. Validation endpoints included tissue viability and preservation of tissue/cell populations in both fresh and frozen/thaw PCLS. Model responsiveness was assessed through controlled exposures to bacterial virulence factors and inflammatory bronchoalveolar lavages (BAL) samples from infected patients, as well as clinically relevant CFTRm.

Results: We established a reproductive workflow enabling the preparation of hundreds of serial slices (within 1-2 working days after lung tissue collection), which can be used fresh or frozen for later experiments, while preserving multicellular architecture (including epithelial, endothelial and connective tissues, and immune cells) and functional features such as ciliary beating. Furthermore, our improved culture conditions for fresh or thawed PCLS allow maintenance of tissue viability for up to 35 days, enabling longitudinal analyses. The protocol also supports controlled application of infectious/inflammatory challenges and pharmacological interventions with CFTRm, along with multimodal outcome measures such as integrity and functional markers, cytokine production/release, and CFTR rescue assessment.

Conclusions: This optimized human PCLS model provides a relevant preclinical platform that maintains the native lung microenvironment for extended functional experiments. It offers a versatile tool for mechanistic investigations of host-pathogen and inflammatory responses in CF, for preclinical evaluation of CFTRm in living tissues, and for dissecting inter-individual variability in therapeutic responses.

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An iPSC-derived airway organoid approach to evaluate potential CFTR modulator benefits in an individual with CF carrying rare compound heterozygous mutations using the FIS assay

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Background: A subset of people with Cystic Fibrosis (CF) remains ineligible to the CFTR modulator therapy due to rare, uncharacterised CFTR mutations. These variants pose challenges to study due to their low prevalence worldwide and limited clinical data. Induced pluripotent stem cells (iPSCs) carrying patient-specific genotypes provide a relevant platform to evaluate CFTR and screen targeted modulators for individualised therapy.
Aims: This study aims to assess CFTR modulator responses using the forskolin-induced swelling (FIS) assay on airway progenitors and basal-like airway epithelial cells (iBCs) derived from iPSCs of an individual with two rare mutations (c.1493_1507del15/1584G>A).
Methods: iPSC lines from a 12-year-old male carrying rare CFTR mutation and homozygous F508del controls were differentiated toward airway progenitors and iBCs. NKX2-1+ airway progenitors and iBCs were enriched using MACS microbeads based on the NKX2-1 surrogate marker carboxypeptidase (CPM) and basal cell marker nerve growth factor receptor (NGFR). The FIS assay evaluated responses to the potentiator VX-770 alone, the triple-combination therapy ETI (VX-445, VX-661, and VX-770), and the potential additive effects of PDE4 inhibitor, Roflumilast.
Results: All lines generated high expression of NKX2-1+ airway progenitors (~90%) and NGFR+ iBCs (~90%). The FIS assays showed that F508del swelled ~50% with ETI in NKX2-1 airway progenitors, ~30% in iBCs and no response to VX-770 alone. The rare mutation line showed no response to VX-770 but responded to ETI (~40% NKX2-1 airway progenitors, ~20% iBCs). Addition of Roflumilast did not further enhance swelling. At lower forskolin concentrations, the rare mutation line suggested no residual CFTR function. RT-qPCR revealed no significant changes in CFTR expression following treatment. Air-liquid interface (ALI) cultures are currently being optimised to extend these observations to fully differentiated airway epithelia, enabling comprehensive evaluation of CFTR modulators in rare mutation line.
Conclusion: Efficient and reproducible generation of airway progenitors and iBCs from CF iPSCs was demonstrated. Our preliminary data revealed that ETI may offer potential benefits for CF caused by rare mutations, with proportional responses observed across cell types.

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Advanced endometrial organoid models to unravel endometrium-linked fertility problems in cystic fibrosis

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Background: Women with CF (wwCF) experience notable fertility challenges, showing higher rates of infertility compared to healthy women (35% *vs.* 5-15%). Since the introduction of highly effective CFTR modulator therapy, pregnancy rates have increased in wwCF. However, underlying causes of fertility problems in wwCF and effects of CFTR modulator therapy on the female reproductive system remain poorly understood. In particular, the pathobiology of the endometrium, the uterus' inner lining essential for embryo implantation toward successful pregnancy, remains largely unexplored in CF. Recently, our lab developed organoids from the endometrium of wwCF (CFEO), which were found to display CF characteristics, interesting transcriptomic differences with healthy endometrium-derived organoids (HEO), and rescue responses to CFTR modulators.
Aims and Methods: In the present study, we employ CFEO to establish advanced models to explore potential aberrations in reproductive processes including embryo implantation and sperm capacitation in CF. The models are compared to the healthy condition, and the impact of CFTR modulators is explored. The early stages of implantation are assessed using our previously developed *in vitro* embryo implantation model, consisting of stem-cell derived blastocyst models (blastoids) which are added on top of EO-derived 'open-faced endometrial layers' (OFEL) with which the blastoids physiologically interact. In addition, we are establishing an endometrium-sperm transwell co-culture system to analyze the impact of endometrial CFTR dysfunction (resulting in altered pH and ion transport) on sperm motility and capacitation, a process needed to render sperm capable of egg fertilization which *in vivo* occurs in the uterus.
Results: The first step of embryo-endometrium interaction, i.e. attachment of the blastoid to the OFEL, was found to be significantly reduced in CF *versus* healthy (fertile) condition. Our current analyses explore whether specific molecular pathways previously identified in CFEO contribute to this impaired implantation and whether CFTR modulators can restore this deficient interaction. Interestingly, preliminary findings indicate an increase in blastoid attachment to CF OFEL when treated with Elexacaftor+Tezacator+Ivacaftor (ETI).
To investigate the impact of CFTR dysfunction in the uterine environment on sperm cells, we are currently comparing the pH in CFEO *vs.* HEO using a pH-sensitive probe and FLIM microscopy. To assess ion transport in CF *vs*. healthy OFEL, we apply the Ussing chamber assay. Our first data reveal elevated ENaC activity and lower CFTR activity in CF, both of which appear normalized by ETI, thereby validating the applicability of the OFEL model to examine ion transport across the endometrium in CF.
Conclusions: By leveraging organoid-derived models, our work will advance mechanistic understanding of CF-associated reduced fertility. Given that CFTR modulators extend life expectancy and increase reproductive opportunities in people with CF, deeper insights are considered very timely and clinically relevant, informing future strategies to support family planning of wwCF.

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Organotypic in vitro model of the human lung using hiPSCs to study epithelial-macrophage interactions and bacterial infections of the upper respiratory tract in cystic fibrosis

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Model systems for the respiratory tract are essential for reliable cystic fibrosis (CF) research and so far rely on animal studies, primary or immortalized cells. However, these approaches fail to capture the complexity of lung physiology while maintaining scalability and relevance to the human disease phenotype. To address these limitations, our group developed a CF lung model using induced pluripotent stem cell (iPSC) technology. Despite the advantages hiPSCs provide, a lack of complex in vitro models that combine respiratory epithelial cells with components of the immune system, particularly macrophages, remains. To bridge this gap, this study seeks to build and characterize an organotypic in vitro model for the lung (AIRMAC) using hiPSC-derived airway epithelial cells (iAECs) and thereof derived Air-Liquid-Interface (iALI) cultures in combination with hiPSC-derived macrophages (iMacs), both carrying the CF genotype and its isogenic control to investigate intrinsic and extrinsic contributions of the CFTR mutation on macrophage and airway epithelial functionality. AIRMAC combines two highly differentiated systems, closely resembling their primary cell counterparts at molecular and functional levels while enabling long-term survival of both cell types for at least 25 days. Within AIRMAC, macrophages exhibit lateral mobility and cluster formation on the iAEC layer, behaviors analogous to in vivo macrophage activity. Current efforts focus on assessing the plasticity and functionality of iMacs in the AIRMAC system including transcriptional and secretome analyses as well as studies on the pathogen and mucus uptake in a time dependent manner. Our lung model is providing a scalable platform for investigating bacterial infections of CF patients in a human-relevant context.

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Implementation of *in vitro* and *ex vivo* approaches to study the impact of CFTR dysfunction on osteocytes in CFBD

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Objectives: Cystic fibrosis-related bone disease (CFBD) occurs in 20-50% of adults with cystic fibrosis (CF). CFBD patients exhibit 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. While the effects of CFTR dysfunction on the bone forming osteoblasts [Delion, 2016; Dumortier, 2025] and the bone resorbing osteoclasts [Jourdain, 2021] are documented, its impact on osteocytes, the sentinels of bone integrity and mineral homeostasis, remains unknown. Osteocytes abnormal behavior has been described in aging [Tiede-Lewis, 2017] and osteoporosis [Sharma, 2012], evidencing modifications of osteocytes lacunae, decreased number of canaliculi per osteocytes, and their interconnections. The aims of this work are, i) to use an innovative *in vitro* model to study the impact of CFTR dysfunction on osteocytes differentiation and function, 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=6 independent donors) were cultured on macrotextured bioceramics for 28 days [Juignet, 2017], without or with CFTR pharmacological inhibitors (Inh_{172}, BPO-27). *In vitro*-formed tissue was investigated by Phalloidin-AlexaFluor® 488-DAPI staining. Osteoblast-to-osteocyte transition was analyzed by RT-qPCR (*BGLAP*, *SPARC, TNFRSF11B, RUNX2, COL1A1, KERA, PDPN, SOST*, *DMP1*, *FGF23*) and ELISA (SOST, FGF23). Eight-week-old CF (n=9 per group) or non-CF (n=6 per group) mice were subjected to OVX (to mimic post-menopausal osteoporosis) or sham surgery. Tibiae were collected, fixed and stained in a solution of absolute alcohol with rhodamine 6G, cut and tissue-cleared for confocal microscopy to qualify and quantify the osteocyte lacuno-canalicular network (LCN).
Results: Initial data showed dense actin network after Phalloidin-AlexaFluor® 488-DAPI staining in bioceramics grooves. CFTR inhibitors did not influence nor on cells viability, neither on cells count, but seemed to modify their repartition inside the grooves. Gene expression pattern analysis coupled with SOST secretion in culture supernatants suggested concomitant mature osteoblasts and osteoid osteocytes inside our cultures. The effect of CFTR inhibitors on osteocyte formation and phenotype require further clarification. OVX surgery evidenced blunted bone microarchitecture in CF condition as compared to wild type and sham counterparts. First confocal imaging of bone samples showed different osteocytes lacunae distribution in mineral matrix, depending on the anterior, posterior, medial or lateral locations, in OVX *versus* sham samples across phenotypes, though total lacunae count remains similar.
Conclusion: Our models seem relevant for investigating osteocyte roles in CFBD. Future work using iPSC-derived MSC will allow us to gain insights on how
native *CFTR* mutations and channel inhibition affect osteocyte involvement in bone homeostasis in CF patients. In parallel, osteocyte LCN analysis will be implemented in an age-related bone loss model.

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Modeling Pseudomonas aeruginosa biofilm and phage therapy in a cystic fibrosis *ex vivo* pig lung model

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Background and Rationale: Colonization by *Pseudomonas aeruginosa* (*Pa*) drives persistent biofilm infections in the lower airways of people with cystic fibrosis (pwCF). Despite repeated antibiotic administration, episodes of acute pulmonary exacerbation occur. Yet, there is still a lack of standardized models reproducing the features of *in vivo* mature biofilm. Most available *in vitro* models rely on abiotic surfaces and therefore offer limited translational relevance for developing alternative therapies.
Hypothesis and objectives: This three-year project is part of a broader research effort to advance phage therapy as a personalized therapeutic strategy for antibiotic-refractory *Pa* chronic lung infections in pwCF. In this perspective, there is a critical need for biofilm models that reliably recapitulate the physicochemical conditions of CF airways and support the development of clinically meaningful biofilm structures.
Essential methods: We established the ex vivo pig lung (EVPL) CF model, which closely mimics the environment of human CF airways. Swine bronchial tissue is infected with *Pa* and incubated with artificial sputum medium reproducing the composition of CF airway secretions and subsequently challenged with laboratory and clinical *Pa* strains, allowing the growth of thick and clinically relevant biofilm structures. The anti-biofilm potential of phage preparations is then assessed using quantitative biomass (e.g., bacterial load) measurements and qualitative analyses by electron and fluorescence microscopy.
Results: We isolated and characterized natural phages specific for *Pa*. A 4-phage cocktail (CKφ) resulted capable of counteract an acute *Pa* infection in some preclinical models of mouse, *galleria mellonella* and CF zebrafish embryo. In the EVPL model, phage treatment almost completely eradicated biofilm formed by laboratory (PAO1) and clinical (LESB58) strains, disrupting the architecture and strongly inhibiting matrix production. Phages showed synergistic effects with commonly used antibiotics as colistin and tobramycin. Not all phages that infected *in vitro* succeeded in eradicating bacterial biofilm. Although the effect on *Pa* isolates from ETI-treated patients suggests a possible shift in phage/antibiotic susceptibility compared to strains from untreated individuals, phage preparations was able to eradicate biofilms of clinical isolates regardless of patient ETI therapy status. Additionally, biofilm formed by phage-resistant mutants (e.g., defective in LPS biosynthesis) was more sensitive to phages exploiting alternative bacterial receptors for the infection, overcoming resistance adaptations.
Conclusions: The study highlights the EVPL CF model as a robust tool to assess phage activity against *Pa* biofilm. Given the persistence of *Pa*, the development of effective phage-based strategies holds strong clinical relevance for pwCF. Furthermore, the implementation of this cost-effective, easy-of-use CF biofilm model may help accelerate the introduction of phage therapy towards personalized clinical application

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Addressing cystic fibrosis inequality in low and middle income countries: a role for patient-derived intestinal organoids

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Background: Over the past decade, cystic fibrosis (CF) care has been transformed with the development of CFTR modulator treatments (CFTRm), which target the underlying protein defect rather than symptoms. These therapies have significantly improved survival and quality of life for individuals with eligible genotypes. However, striking global inequities persist. In high-income countries, median life expectancy for people with CF (pwCF) exceeds 65 years, whereas in many low- and middle-income countries (LMICs) children often die undiagnosed in early childhood. Major challenges in LMICs include diagnostic capacity, patient registration, and treatment access. Patient-derived intestinal organoids (PDIOs) may help in addressing these gaps.
Methods: We review recent literature across three domains: (1) CF-related challenges in LMICs; (2) the potential of PDIOs to address these gaps: and (3) the obstacles to their implementation in LMICs. The review was developed collaboratively with LMIC researchers and global health equity experts in CF care to ensure inclusion of diverse clinical and healthcare perspectives.
Results: PDIOs, cultured directly from patient tissue, preserve the individual’s genomic context and enable quantification of CFTR function, which correlate with disease severity and modulator responsiveness. They provide a powerful tool for genotype-phenotype characterization, personalized modulator screening, and identification of potential responders with rare or unclassified CFTR variants. PDIOs also support international collaboration, as rectal biopsies can be cryopreserved, shipped, and expanded, facilitating centralized testing hubs. Estimated per-sample costs (€1500-€4000) suggest a cost-effective means to optimize modulator use and reduce unnecessary treatment of non-responders.
Conclusion: PDIOs represent a promising, scalable model to help close diagnostic and therapeutic gaps in CF care globally. However, their implementation in LMICs remains constrained by limited validation, technical capacity, and access to CFTR modulators. Strengthened international partnerships, technology transfer, and sustainable funding will be essential to realize their full potential in advancing equitable CF precision medicine.

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Cystic fibrosis associated colorectal cancer: linking CFTR-deficiency to the increased risk of developing CRC

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Background: Life expectancy in people with cystic fibrosis (pwCF) has significantly improved because of advances in clinical care and the introduction of CFTR modulators. However, recent studies indicate that ageing pwCF experience age-associated comorbidities, including an increased risk of developing colorectal cancer (CRC). PwCF aged 40-49 show a CRC prevalence of ~23%, compared to 2-5% in similarly aged non-CF individuals.
Understanding how CFTR deficiency affects intestinal epithelial biology, particularly its potential tumor-suppressive roles, is essential for improving CRC prevention and treatment in this population.
Therefore, we aim to investigate cell-autonomous mechanisms involved in CFTR loss-of-function (LoF) for the development of CRC. In parallel, we characterized polyp incidence in the CF population and established a biorepository of polyps from CF and non-CF patients to validate mechanistic findings in clinically relevant tissue.
Methods: We exploited previously generated non-isogenic patient-derived intestinal organoid (PDIO) cultures across a range of CFTR genotypes and generated CRISPR-engineered isogenic CFTR-KO organoid pairs. Proliferation and growth factor dependency were quantified under varying Wnt conditions. Additionally, PDIOs homozygous for *F508del* were treated with or without CFTR modulators (Elexacaftor/Tezacaftor/Ivacaftor). To identify pathways altered by CFTR-LoF in an unbiased manner, we performed bulk RNA sequencing followed by ongoing validation using qPCR, western blotting, and Luminex.
Clinical data from pwCF treated at the UMCU were analyzed for polyp incidence, dysplasia type, age, and modulator use. A biorepository of CF and non-CF polyps obtained during colonoscopy was established and will be used for spatial transcriptomics.
Results: In complete organoid medium, CFTR-KO organoids displayed reduced growth relative to its isogenic wild type. Under reduced Wnt conditions, however, CFTR-KO organoids demonstrated a proliferative advantage, revealing diminished Wnt dependency as a direct result of CFTR-LoF. In *F508del/F508del* PDIOs, ETI treatment enhanced growth compared with untreated cultures. In reduced Wnt conditions, untreated *F508del* organoids displayed higher proliferation, mirroring the phenotypes observed in isogenic CFTR-KO models. These findings indicate that CFTR activity could influence epithelial growth control and Wnt pathway sensitivity. Bulk RNA sequencing revealed reduced expression of the Wnt inhibitor *DKK1* under CFTR-LoF conditions, providing a potential mechanistic link between CFTR deficiency, altered Wnt signaling dynamics, and increased proliferative capacity.
Clinical cohort analysis is expected to be finalized in January 2026.
Conclusion: This work establishes a mechanistic framework connecting CFTR-LoF to CRC susceptibility and highlights epithelial organoids as a powerful system to explore CFTR-dependent tumor-suppressive pathways. The complementary clinical dataset and biorepository will further strengthen the translational impact of these findings.

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Cystic fibrosis sputum enhances neutrophil motility in 3D collagen matrices

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Early, massive, and sustained neutrophil-driven inflammation is a hallmark of Cystic Fibrosis (CF) airway disease, leading to chronic tissue damage. Neutrophil dysfunction in CF is remarkably restricted to the lungs, suggesting the involvement of lung-intrinsic factors in modulating their behavior. However, whether the CF lung microenvironment directly regulates neutrophil dynamics is poorly characterized.
Here, we investigated the role of CF lung components in the regulation of neutrophil migration ex vivo. Using state-of-the-art microfabrication and image processing, we tracked the impact of sputum from CF patients and healthy donors on neutrophil migration within a 3D collagen matrix in real time.
Single-cell trajectory analysis showed that CF sputum significantly increased human primary neutrophil migration speed compared to healthy donors (8 µm/min vs. 4.25 µm/min, respectively). This phenotype was accompanied by higher displacement and a faster response onset. Furthermore, employing an interstitial-space-on-a-chip device, we demonstrated that this enhanced motility is a chemotactic response in h-Neutrophils induced exclusively by CF sputum. Crucially, the absence of CFTR in CF patient neutrophils did not affect their 3D migratory capacity or chemotactic response.
To identify potential mediators, we assessed the contribution of IL-8, IL-6, and IL-17, known to be elevated in CF airways. However, none of these stimuli reproduced the prolonged migratory effect observed with CF sputum.
Collectively, our data reveal that soluble factors in CF sputum potently enhance neutrophil motility in 3D collagen, highlighting a potential role for epithelial secretions in modulating neutrophil dynamics in the diseased lung. We are currently defining the nature of these molecules, which represent critical new targets to reduce neutrophil recruitment in CF, alongside evaluating the impact of Kaftrio therapy on this sputum-driven effect.

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Illuminating CF drug discovery via fluorescent organoid-derived screening models

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CFTR modulators have transformed Cystic Fibrosis (CF) care, yet variability in individual responses highlights the need for more predictive and physiologically relevant drug discovery assays. Patient-derived intestinal organoids (PDIOs) enable high-throughput (HT), but indirect, assessment of CFTR function via the Forskolin-Induced Swelling (FIS) assay [1]. In contrast, genetically encoded ion-sensitive fluorescent reporters—such as HS-YFP-mKate for iodide flux [2] or ClopHensor for chloride and pH sensing [3]—provide direct, quantitative, real-time measurements of CFTR channel behaviour. Integrating these sensors into highly predictive primary intestinal models may therefore enhance functional resolution and provide more robust, physiologically relevant readouts, improving drug discovery methods for multiple CFTR variants.
To address this, we aim to establish and validate fluorescence-based assays capable of directly reporting CFTR-dependent chloride, bicarbonate and pH dynamics in PDIO-derived systems. HS-YFP-mKate and Clop3 (ClopHensor derivative) constructs were cloned into lentiviral backbones via Gibson Assembly, packaged in HEK293T cells and used to transduce WT and F508del/F508del PDIOs. Reporter-positive organoids were subsequently expanded and purified through manual selection and FACS sorting. Both 3D and 2D assay formats are under development to support automated high-throughput imaging and analysis.
We successfully generated stable fluorescent PDIO populations expressing HS-YFP-mKate and Clop3. Preliminary measurements demonstrate iodide-dependent HS-YFP quenching following CFTR activation, and optimisation of Clop3 imaging protocols for combined chloride and pH quantification is ongoing. These emerging models offer a platform for direct CFTR function evaluation, with potential to enable scalable screening and improve prediction of patient-specific drug responses.

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From Cells to Breath: Identifying therapeutic biomarkers using *in vitro* volatilomics

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Background: Recent clinical studies have shown that the composition of exhaled breath is modified in people with CF initiating CFTR modulator therapies [1,2]. Significant volatile organic compounds (VOCs) have been identified in breath; yet, the metabolic pathways leading to their generation, and how these are impacted by treatments, remain to be explored.
Methods: To facilitate the study of VOCs released by respiratory cells, we have built a unique chamber that supports the culture of cellular models *in vitro* whilst capturing the VOCs produced by cells. Three batches of human bronchial epithelial F508del BMI-1 cells were cultured at the air-liquid interface and treated with elexacaftor-tezacaftor-ivacaftor (ETI) or vehicle (DMSO) for 48h. Additional controls were included in the experimental plan, including culture medium and BMI-1 cells with CFTR-WT and G542X genotypes, exposed to DMSO or ETI. VOCs were extracted from cell cultures after medium renewal and concentrated onto Tenax® tubes; VOCs were then released by thermo-desorption and analysed by mass spectrometry (TD-MS). Multivariate statistical analyses were applied to identify potential ETI-associated VOCs.
Results: The batch influenced the detected VOC profiles, indicating that these experiments are highly sensitive and require appropriate parallel controls. Nine VOCs were significantly modulated by ETI in F508del cells, including one that was also increased in WT cells. These VOCs are currently being characterised to study their biological origin. Furthermore, we will assess whether the *in vitro* VOCs correspond to breath VOCs altered by ETI in patients, as suggested by preliminary findings on CF primary cells. Differences in volatilomic profiles between genotypes will also be investigated.
Conclusions: *In vitro* volatilomics may reveal ETI-associated metabolites that may serve as non-invasive therapeutic biomarkers *in vivo*, and bring new insight into modulators' mode of action.

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Using air–liquid interface airway cultures to study early *Pseudomonas aeruginosa* infection behaviours during CFTR modulator therapy

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Background: Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) modulators have changed the clinical course of cystic fibrosis (CF), but it is still not well understood how the altered epithelial environment influences the earliest steps of *Pseudomonas aeruginosa* (PA) colonisation and infection. Air–liquid interface (ALI) airway cultures offer a controlled model system that retains many features of the human airway, making them well suited for studying early infection events. In this project, we used ALI cultures to examine how PA strains behave on epithelial surfaces exposed to the highly effective modulator elexacaftor/tezacaftor/ivacaftor (ETI).
Methods: Primary nasal epithelial cells from CF and non-CF donors were expanded and differentiated into ALI cultures. After 24 hours of ETI treatment, cultures were inoculated with PAO1 and selected clinical isolates. Alongside standard infection readouts including CFU counts, transepithelial electrical resistance (TEER), LDH release, and IL-8 secretion, we used confocal microscopy to visualize how bacteria positioned themselves on the epithelial surface, interacted with mucus, and initiated early microcolonies.
Results: At baseline, epithelial barrier measurements clearly distinguished CF from non-CF cultures, with CF cells showing consistently higher TEER values prior to any intervention. After ETI treatment, TEER decreased in the CF cultures compared with untreated CF cells and non-CF controls. Despite these changes in epithelial integrity, ETI did not alter the infection outcomes. Across PAO1 and the two clinical isolates, CFU counts, TEER responses during infection, and IL-8 secretion remained similar between ETI-treated and untreated cultures, regardless of donor background.
In contrast, imaging analyses revealed more nuanced differences in PA colonisation behaviour that were not captured by quantitative assays. The bacterial strains exhibited distinct spatial behaviours on the epithelial surface, including variation in their distribution and interaction with the mucus layer. These patterns reflected inherent strain characteristics rather than effects driven by ETI treatment. Confocal microscopy also showed a marked increase in apical CFTR localisation after 24 hours of ETI exposure
Conclusions: We did not observe differences in infection outcomes between ETI-treated and untreated cell cultures. Confocal microscopy confirmed increased apical CFTR expression after 24 hours of ETI exposure in CF cells and showed clear strain-dependent patterns of bacterial localisation. These findings support the use of ALI epithelial cultures as a valuable model for studying early bacterial infection dynamics. Incorporating immune cells into the system may further enhance its relevance and improve our understanding of airway colonisation and infection processes.

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Modeling class I mutation with CRISPR/Cas tools: hiPSC-derived organoids in cystic fibrosis

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Lung damage remains a key feature of the Cystic Fibrosis disease. The triple therapy Kaftrio ® reduces lung symptoms and offers a promising therapy for approximately 80% of patients. This paved the way for intensified research into the discovery of drugs for rare mutations (e.g., Class I).
Circumventing the defects caused by class I mutations is still a challenge for the development of effective therapeutic strategies, and this, in turn, relies on the availability of a suitable cellular model.
Differentiated CRISPR/Cas-edited iPSCs yield isogenic airway models of CF.

CRISPR/Cas genome editing; iPSC clones characterization; Bronchial epithelium production and characterization (directed differentiation, epithelial cell types identification and quantification by IF and FACS, ciliary beat frequency); CFTR function (airway organoids (AOs) size quantification, FIS assay).
We successfully generated five iPSC lines homozygous for CFTR mutations: (1) two premature stop-codon (c.1657C>T, c.366T>A), (2) two intronic mutations (c.1585-1G>A, c.1680-886A>G), (3) and a deletion in phase mutation (c.1521-1523del). We used specific Cas depending on the sequence context. These iPSC lines show conservation of pluripotency and genome integrity. Pseudostratified epithelia c.1521-1523del and c.1680-886A>G are composed of the main cell types found in *in vivo* bronchus. AOs bearing c.1521-1523del, c.1657C>T, c.1585-1G>A, c.366T>A, and c.1680-886A>G mutations are lower in size than the WT isogenic AOs. FIS assays demonstrated that organoids carrying the c.1521_1523del and c.1680-886A>G mutations failed to swell. However, treatment with Kaftrio® restored swelling in c.1521_1523del organoids.
iPSC-derived bronchial epithelia recapitulate CF phenotypes with robust functional readouts, offering valuable models for the development of class I CFTR therapies.

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Of mice and men: Decoding the genomic background of βENaC-Tg mouse model through whole genome sequencing

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Cystic Fibrosis (CF) is a debilitating disease primarily affecting the airway and gut of over 100,000 people worldwide. Many people with CF (pwCF) have dehydrated airway surface liquid (ASL) and mucus plugging. However, many of the CF animal models do not mimic the phenotype observed in pwCF, which can impair assessment of the true efficacy of developed delivery strategies of gene editing approaches *in vivo*. To circumvent the limitations of most conventional CF animal models, we chose the CCSP promoter-driven β-ENaC over-expression mouse model which resembles more closely a CF human phenotype. These transgenic mice were originally generated via microinjection of the mouse oocyte with the β-ENaC expression cassette, selectively bred producing a hemizygous mouse line and transgene presence was confirmed via genotyping[1]. However, the precise location and copy number of the transgene has not been reported.
Here, we aimed to further characterize the β-ENaC mouse model to elucidate the exact location of the integration site and the copy number and sequence integrity of the CCSP promoter-driven β-ENaC gene that are present in the model. To do this, we performed whole-genome sequencing (WGS) of the β-ENaC mouse model. Genomic DNA was extracted from mouse tail snips via digestion and subsequent ethanol precipitation, and the genotyping PCR was performed on the extracted gDNA samples to confirm the correct hemizygous genotype. Whole Genome Sequencing (WGS) was performed by Oxford Nanopore (ONT) Long-Read Sequencing with the CCHMC Genomics Sequencing Facility, as well as by NovoSeq X Plus short-read WGS sequencing with Genewiz from Azenta Life Sciences.
Preliminary data analysis indicates that there is a single insertion site of the gene of interest at chromosome 5 with at least one fully intact copy of the β-ENaC expression cassette. Further analysis will establish the exact number and direction of fully functional copies through analysis of the acquired WGS data, Oxford Nanopore Technologies sequencing long-range PCR spanning large regions of interest, and qPCR to assess vector copy number. This acquired data will allow us to determine the suitability of the engineered β-ENaC mouse model as to test gene editing delivery strategies for correction of CF-causing mutations.
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A unique combination of nucleotide modifications enhances suppressor tRNA efficacy in restoring CFTR function

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Approximately 10% of patients with cystic fibrosis harbor a nonsense mutation in the CFTR gene that confers a premature termination codon (PTC). This type of molecular defect results in the abrupt arrest of translation and loss of protein function. To correct PTCs, we have developed a novel strategy that repurposes sense-codon-decoding tRNAs into suppressor tRNAs (sup-tRNAs). These sup-tRNAs read through PTCs, thereby restoring full-length CFTR protein and functional activity (1, 2). We pursue two administration strategies: single-dose administration using episomal vectors and redosing of ready-to-use sup-tRNAs delivered by lipid nanoparticles. To minimize the need for redosing, we aim for maximum efficacy and intracellular stability of the sup-tRNAs. We identify a unique nucleotide modification that enhances the suppression activity of various sup-tRNAs up to 5-fold. We combine this modification with a secondary modification in the D-arm of the sup-tRNA, and show these changes can efficiently silence immunogenicity and blunt the innate immune response following sup-tRNA administration. The modified sup-tRNAs exhibit high stability, improving the therapeutic half-life. Modified sup-tRNAs restore CFTR activity of several disease-causing PTCs to levels above a threshold predicted to be therapeutic. In the single-dose approach, we modify the promoter-terminator tandem to achieve sup-tRNA levels comparable to the tissue-specific levels, causing no perturbation of the natural tRNAs. Notably, in difficult-to-suppress PTCs, such as G542X, we propose an alternative strategy, achieving substantial activity of CFTR in the FRT model – a cell system viewed as relevant to drug registration by the FDA.

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Safely skipping stops: a proteomics-based framework for evaluating readthrough therapies in cystic fibrosis

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Background and Objective: Roughly 10% of all people with cystic fibrosis (pwCF) carry nonsense mutations that introduce premature termination codons (PTCs) in the CFTR gene, resulting in truncated, non-functional protein. Readthrough-based therapies, such as small molecules and anticodon-edited tRNAs (ACE-tRNAs), can restore CFTR synthesis, but clinical translation is so far unsuccessful. A reason for this is the efficacy-safety trade-of, where a side-effect of readthrough strategies could be the potential suppression of natural termination codons (NTCs), leading to C-terminally extended proteins with unknown effects. A technique used in some previous studies to characterize safety of readthrough strategies is ribosomal profiling, which provides partial insights by mapping ribosome positions on mRNA. However, it fails to assess whether translated proteins are stable or functional, which is critical to understand safety of readthrough small molecules and ACE-tRNAs. To address this gap in knowledge, we propose a mass spectrometry-based proteomics approach coupled with bioinformatics analysis pipeline to systematically assess NTC fidelity at the protein level.

Methods: Patient-derived intestinal organoids (PDIOs) and human bronchial epithelial (HBE) cells with CFTR nonsense mutations are treated with small-molecule (ELX-02, DAP) and ACE-tRNA readthrough agents. CFTR functional rescue is quantified using forskolin-induced swelling and Ussing-chamber assays. Parallel mass-spectrometry proteomics profiles proteome-wide alterations and possible NTC readthrough events, integrated through dedicated bioinformatic pipelines linking functional and molecular outcomes.

Results: Preliminary proteomic analyses confirm absence of C-terminally extended proteins upon ELX-02 and ACE-tRNA treatment in the PDIOs. While proving the *absence* of NTC readthrough is inherently challenging, pilot data provide strong leads that this can be robustly assessed within our workflow, e.g. the detection of several C-terminally elongated protein variants that are known to be produced in in normal cell physiology. Detection of this and other positive control protein variants, support the feasibility of a proteomics-based quantitative “safety fingerprint” for each readthrough strategy. At the time of the Basic Science, we expect to have conducted first complete comparison experiments with the different readthrough strategies in the different cell models.

Conclusions: Our study establishes a scalable and sensitive proteomic framework to balance efficacy and safety of readthrough-based therapies, from small molecules to ACE-tRNAs. This approach directly addresses a key translational barrier for pwCF carrying nonsense mutations and can be extended to other genetic diseases caused by premature stop codons.

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Silencing the silencers: antisense oligonucleotides targeting intronic splicing silencer motifs in CFTR

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Introduction: Among the ~2,100 reported potentially disease-causing CFTR variants, over 10% disrupt pre-mRNA splicing [1]. Yet the regulatory architecture driving these events - especially intronic splicing silencers (ISSs) and their RNA-binding protein (RBP) binding sites - remains poorly defined. Steric-blocking ASOs can restore exon inclusion by preventing repressive RBP-RNA interactions [2]. Our previous rescue of c.2657+5G>A by targeting putative downstream intronic elements suggested ISS interference as the mechanism, but the responsible motif was unknown [3]. Here, we map ISS motifs across CFTR and assess their value as therapeutic entry points for loss-of-splicing (LoS) variants.
Methods and results: We compiled a unified ISS motif set by integrating SpliceAid-F predictions with experimentally validated intronic silencers from the literature. Using this library, we mapped candidate and validated ISS motifs across the CFTR locus. We next assessed 328 CFTR single nucleotide variants with SpliceAI and MaxEntScan to predict LoS variant effects and scored each variant for integrity of its donor/acceptor site. Combining these metrics, we developed a prioritisation framework for the identification of variants most likely to respond to an ISS-blocking ASO strategy. This classified 141 variants as LoS and highlighted 50 with the highest predicted amenability - the highest ranked of which was the recurrent c.2657+5G>A. Local motif mapping around the intron 16 5’ splice site revealed five experimentally validated ISS motifs in proximity to this variant. To test functional relevance, we co-transfected HEK293T cells with a CFTR c.2657+5G>A mini-gene and ASOs targeting the ISS motifs. Here we present data on restoration of exon 16 inclusion by these ASOs, and the extent to which steric inhibition of this site can rescue the splice defect associated with c.2657+5G>A.
Conclusions: We provide the first integrated map of intronic splicing silencer motifs across CFTR and identify a subset of LoS variants with therapeutic potential for ASOs blocking nearby ISS elements. Mechanistic validation in c.2657+5G>A mini-gene model suggests that targeting motif-rich regions can restore exon inclusion. Further motif-level mutagenesis will be used to refine this mechanism; however our preliminary evidence supports ISS-blockade as a viable correction strategy.
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Pro-repair strategies targeting CFTR and K+ channels and counteracting bacterial virulence to restore airway epithelial integrity in cystic fibrosis

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Background: Pulmonary disease remains the major cause of morbidity and mortality in cystic fibrosis (CF) and the recovery of lung function depends on efficient repair of airway damage, particularly in individuals with advanced disease. Beyond its essential role in regulating ion and fluid transport, necessary for mucociliary clearance, CFTR also contributes directly to epithelial repair. Therefore, both the CFTR defect and the presence of chronic infection impair the capacity of the CF airway epithelium to repair effectively after injury. Pro-repair therapeutic strategies are thus needed to restore lung integrity.
Previous work from our group showed that functional rescue of CFTR using modulators, as well as complementary approaches targeting K^+ channels or reducing bacterial virulence, can promote epithelial repair.

Objective: We aimed to dissect how CFTR regulates epithelial repair and to determine how CFTR modulators improve early and late repair processes. We also sought to evaluate combined approaches involving CFTR modulation, K^+ channel activation, and strategies designed to attenuate bacterial virulence.

Methods and results: Early and late repair processes (including cell migration and proliferation of progenitor cells, repair/regeneration of an intact, differentiated, ciliated epithelium) were assessed using primary airway epithelial cell cultures derived from people with CF (pwCF) carrying a range of CFTR mutations. Our results confirm that epithelial repair is impaired in CF. Functional rescue of CFTR with modulators (Lumacaftor/ Ivacaftor or Elexacaftor/Tezacaftor/Ivacaftor) improved guided cell migration at the wound-edge, associated with activation of key proteins regulating the cell migration process. CFTR rescue also accelerated wound closure and promoted regeneration of a tight and ciliated epithelium after chronic treatments.
Combined approaches decreasing the virulence of *P. aeruginosa* and *S. aureus* preserved epithelial repair under infectious conditions, supporting the relevance of targeting pathogen-driven impairment of healing. Importantly, activation of K^+ channels exerted significant pro-repair effects in CF cultures, with mutations non-responding to CFTR modulators, demonstrating CFTR-independent pathways can restore epithelial capacity.

Conclusions: Together, these findings demonstrate that CFTR function is central regulator of airway epithelial repair and that functional rescue of CFTR substantially improves both early and late stages of tissue repair. K^+ channel activation and attenuation of bacterial virulence further enhance repair, including in settings where CFTR modulators alone are not efficient. These results support the development of combination therapeutic strategies, adapted to CFTR mutations, aimed at restoring robust epithelial repair in CF, with potential benefits for patients with advanced pulmonary disease or limited responsiveness to current CFTR modulators.
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A cell-permeable nanobody synergizes with ETI to restore near-normal F508del-CFTR function in airway epithelial cells from patients with cystic fibrosis

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Background: The most common cystic fibrosis (CF)-causing mutation, *F508del*, resides within nucleotide-binding domain 1 (NBD1) of the CFTR chloride channel. Although elexacaftor/tezacaftor/ivacaftor (ETI) therapy restores CFTR function to ~50% of normal in individuals carrying at least one *F508del* allele, it does not stabilize the thermodynamically disrupted NBD1, and patients on ETI continue to exhibit persistent airway infection and inflammation. Furthermore, natural-history studies indicate that near-normal CFTR function is required to prevent CF-related lung disease. Here, we investigated a novel NBD1-targeting nanobody (NB1) that stabilizes F508del-CFTR and may enhance ETI efficacy, but whose functional impact has remained unclear due to challenges in intracellular delivery.

Aims: (i) Enable efficient intracellular delivery of NB1 into highly differentiated airway epithelial cells from patients with CF using cell-penetrating peptides (CPPs); (ii) define NB1's effects on F508del-CFTR expression, maturation, and function; and (iii) assess its functional synergy with ETI.

Methods: A cysteine-engineered NB1 was conjugated to the CPP R_{10} via a cleavable disulfide bond. CFBE41o- cells expressing F508del-CFTR were treated with NB1-R_{10}, and CFTR function was quantified by transepithelial short-circuit current (I_{sc}) measurements of CFTR inhibitor-172-sensitive currents (ΔI_{sc} CFTRinh-172) following cAMP stimulation. CFTR maturation was examined by Western blotting. Primary airway epithelial cultures from *F508del*/*F508del* patients were used to evaluate NB1-R_{10} uptake and its functional effects on CFTR - alone and combined with ETI. Primary airway epithelial cultures from healthy donors served as wild-type controls for I_{sc} measurements.

Results: R_{10} enabled robust, dose-dependent intracellular delivery of NB1 in CFBE41o- cells. Furthermore, NB1-R_{10} increased F508del-CFTR maturation, apical trafficking, and channel activity. In primary airway epithelial cultures from CF patients, NB1-R_{10} demonstrated efficient uptake and significantly improved CFTR function relative to vehicle (ΔI_{sc} CFTRinh-172 = 2.84 ± 0.19 vs. 1.49 ± 0.07 µA/cm²; $p < 0.001$). ETI alone restored CFTR function to 55% of wild-type levels (ΔI_{sc} CFTRinh-172 = 23.01 ± 1.17 µA/cm² vs. 41.52 ± 5.42 µA/cm²). Notably, concurrent treatment with NB1-R_{10} and ETI resulted in significantly greater functional rescue compared to ETI alone (ΔI_{sc} CFTRinh-172 = 36.85 ± 1.54 µA/cm²; $p < 0.001$), achieving ~89% of normal CFTR function.

Conclusions: This study provides the first evidence that intracellular delivery of an NBD1-stabilizing nanobody can synergize with ETI to restore F508del-CFTR function to near-normal levels in primary airway epithelia from patients with CF. These findings highlight cell-permeable nanobodies as a promising strategy for intracellular protein targeting and the development of next-generation biopharmaceuticals.

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Allele-specific editing in primary human nasal epithelial cells with compound heterozygous genotypes

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Though CRISPR technology has, in principle, the potential to correct many of the cystic fibrosis transmembrane conductance regulator (*CFTR* defects, not all genotypes are equally straightforward to address.

With over 650 identified disease-causing *CFTR* variants, approximately 51% of people with Cystic Fibrosis (pwCF) carry a compound heterozygous genotype; among these, 82% harbour at least one F508del allele — representing around 68'000 people. It is therefore central to therapeutic advancement to target compound heterozygotes, as correcting or functionally rescuing even one of their pathogenic alleles could, in effect, shift an individual towards a carrier-like phenotype with markedly improved CFTR activity.

As complex genotypes may necessitate distinct combinations of modulators or gene-editing approaches, a flexible, broader strategy that can be tailored to address the specific variants, or regions where these present in clusters, would expand therapeutic possibilities. Building on our previous plasmid-based transfection studies to edit CFTR variants in IB3-1/S9 and 16HBEge heterozygous cell lines, here we describe the application of an RNA-based delivery approach to correct the F508del variant (exon 11) in patient-derived primary cells also c.53+1093_53+1094ins (intron 1) as the compound heterozygous variant. Our strategy exploits a Cas9 nickase and two sgRNAs to trigger allele conversion around the mutation site availing of the respective exon 11 wild type sequence in the homologous chromosome as the donor template. Specifically, the correction is driven by a primary, allele-specific sgRNA that creates a nick only on the allele with the target mutation, and a secondary sgRNA creating an allele-agnostic nick on both. The editing is measured by Next Generation Sequencing as conversion of a mutant allele into wild type allele for the target region relative to the variant's sequence.

In our initial studies we observed a 0.92% increase of wild type exon 11 in cells treated with Cas9n and the two sgRNAs relative to the transfection control. This result is in line with what was achieved using the same sgRNA combination in16HBEge F508del/WT – with a 5.99% increase in wild type exon 11 – and our preliminary experiment with a less efficient sgRNA combo in IB3-1/S9 cell line (F508del/W1282X) — reporting a 0.47% increase of wild type exon 11.

Future studies will focus on improving transfection efficiency, and carrying out functional assays, along with further exploring patient-derived samples. With most variants being clustered in key regions of the *CFTR* gene, it is imperative to investigate how nicking in close proximity to both mutations affects the editing outcome.

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

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Background: Several CFTR-targeted therapeutic strategies have been approved, and the number of *CFTR* mutations addressed by these agents is increasing. Nevertheless, around 20% of patients remain ineligible for CFTR modulators, particularly those carrying splicing mutations (~12% of the reported CFTR mutations). We specifically focused on deep intronic mutations that induce aberrant splicing patterns, most often through the insertion of an intronic region into the mRNA sequence (cryptic exon, CE), generally leading to the creation of a premature termination codon.
Aims: We used an approach based on modified oligonucleotides (Target Site Blockers, TSBs), to specifically target and block aberrant splicing sites on *CFTR* pre-mRNA (1,2). We designed TSBs against several mutations and assessed their effectiveness in various cell models. Our promising results lead us to optimize TSBs and evaluate their efficiency in more complex models, as well as their *in vivo* distribution.
Methods: TSB optimization involved selecting appropriate chemical modifications on the ribose constitutive of the TSB and choosing an efficient delivery agent. Regarding the first aspect, we selected different chemical modifications already published and used in clinical trials. For each mutation, we designed TSBs containing nucleotides with different chemical modifications (*Locked Nucleic Acid* , LNA ; *O-Methoxyethyl*, OME ; *O-Methyl*, OM), varying their positions and proportions within the TSB sequence. We screened the efficiency of all TSBs in bronchial cells transfected with specific in-house minigenes containing *Firefly Luciferase* gene interrupted by mutated intronic sequences. Luciferase expression, and thus activity, only occurred under normal splicing conditions, allowing us to quantitatively assess TSB efficacy in restoring normal splicing and to compare efficiency of TSBs targeting a same mutation. The most effective TSB was further tested in more complex models (Air-Liquide Interface cultures of human nasal cells and cells derived from hiPSC) under various delivery conditions.
Results: Luciferase assays showed that our chemical optimization enhanced the efficiency of 5 TSBs designed for 5 different intronic mutations. Our results showed that these TSBs were able to block CE inclusion in several deep intronic mutations conditions, increasing normal mRNA splicing by 2 to 6 times (depending on the mutation) in bronchial cell lines. We focused on the TSB against c.1680-883A>G and c.1680-886A>G mutations, and assessed its efficiency on more complex models. We are currently focusing on optimization of its cellular uptake by selecting an appropriate delivery agent and have initiated *in vivo* studies to evaluate its biodistribution.
Conclusions: Our findings demonstrate the efficacy of a TSB-based strategy in restoring normal *CFTR* mRNA splicing. The efficiency of these TSBs was further improved through chemical optimization. This TSB-based approach represents a promising therapeutic strategy for patients with deep intronic mutations.

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Temporospatial regulation of adenine base editing to identify therapeutic target cells in the airway epithelium

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For people with CF (pwCF) lacking a causal treatment, gene-based therapies represent the best possibility for a cure. However, beyond the development of efficient editing and delivery tools, it is not clear which cell types in the airway epithelium need to be edited to restore CFTR function to levels that correspond to clinical benefit.

To find the answer to this fundamental question, we aim to develop a tool that combines a temporal and spatial regulation of gene editing in the airway epithelium. For this, we focused on adenine base editing (ABE) and on W1282X, the second most common CF-causing variant which cannot be addressed by current CFTR modulators.

To achieve temporal control, we developed a SplitABE system in which the Cas9 C-term is fused to FKBP and carries a nuclear localization signal (NLS), whereas the Cas9 N-term is fused to the ABE and FRB, and contains a nuclear export signal (NES). The FKBP/FRB domains enables rapamycin-inducible heterodimerization, thereby restricting editor assembly to desired time windows. For spatial control, the SplitABE/sgRNA cassette was cloned into lentiviral vectors (pLVX_Hygro) downstream of cell-type–specific promoters[1].

Once we obtained the final pLVX_Hygro_Promoter_SplitABE plasmids, we tested the construct carrying the KRT5 promoter (specific for basal cells) in non-differentiated W1282X-BCi cells. We confirmed the expression of Cas9 C-term and N-term by WB with and without rapamycin, as well as the localization of the two halves of Cas9 by IF. As expected, Cas9 C-term is always detected in the nucleus, whereas the Cas9 N-term translocates from the cytosol to the nucleus upon the addition of rapamycin.

To generate novel cell lines with expression of SplitABE, the five constructs obtained were used to transfect the packaging cell line 293T to produce lentiviral particles on a large-scale: W1282X-BCi cells were then transduced with the LV at various MOIs. As hygromycin-based selection was difficult to achieve, novel constructs were produced by replacing the HygroR marker with TurboGFP, thereby enabling direct visualization of packaging and transduction efficiency as well as enrichment of positive cells by fluorescence-activated cell sorting (FACS).

W1282X-BCi were, therefore, electroporated with either pLVX_Hygro_SplitABE or pLVX_TurboGFP_SplitABE. WB results revealed a higher Cas9 expression in the cells transfected with pLVX_TurboGFP_SplitABE, especially upon the addition of rapamycin. These preliminary data suggest that the alternative plasmid may enhance overall editing efficacy.

In summary, this novel gene editing tool combined with the newly generated cell model provides a powerful approach to identify the airway epithelial target cells required for meaningful CFTR correction and to refine strategies for future clinical translation.

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Prime editing of the F508del CFTR variant: balancing epegRNA design and editor optimization

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Objectives: Cystic fibrosis (CF) is most frequently caused by the F508del variant in the *CFTR* gene. Although pathogenic therapies exist for this genotype, they remain imperfect and do not fully address the disease cause, underscoring the need for causal treatments. In this study, we explored prime editing (PE) as a potential tool for correcting F508del. PE enables targeted sequence alterations without generating double-strand breaks and is therefore considered a promising approach for therapeutic genome editing. However, its application to the F508del site is complicated by the AT-rich sequence context. In addition, prime-editing efficiency is constrained by the step in which the edited DNA strand must be effectively retained during flap resolution. To overcome these restrictions, we evaluated two different strategies: rational engineering of guide RNAs for PE (epegRNAs) and modification of the editor itself through fusion with nucleases that may facilitate the removal of the unedited DNA strand.

Methods: We screened 24 epegRNA designs in airway basal airway cells derived from hiPSCs of patients homozygous for F508del. In parallel, we developed12 PEmax-based editor variants fused to FEN1, EXO1 or the EXO1 active domain (HEX-N2) through linkers of different lengths, and examined their performance in the same cellular model. Editing outcomes were quantified by deep targeted sequencing 72 hours post-delivery. Statistical analysis was carried out using Dunn’s test.

Results: Our results demonstrated that carefully optimized epegRNA designs can overcome the limitations imposed by the AT-rich sequence context.

Notably, the most effective epegRNA tailored for PE2-NG – whose Cas9 variant recognizes a more permissive PAM than the one required by PEmax – achieved an editing efficiency of 23.5% on average, despite PE2-NG generally being considered less potent. At the same time, the best-performing modified editor was the PEmax variant fused to FEN1 through a four–amino acid linker, which approximately doubled the correction efficiency compared with unmodified PEmax, reaching 2.13% on average.

Conclusions: We identified prime-editing strategies capable of correcting the F508del variant in patient-derived airway basal cells with efficiencies approaching 24%. Importantly, while nuclease-enhanced editor variants showed measurable improvement, the most decisive factor for achieving high correction rates was the thoughtful design of epegRNAs and the use of PE2-NG. Therefore, optimization of guide-RNA architecture appears to be a more influential parameter than editor modification for F508del targeting, though nuclease-fused systems may remain valuable for other genomic loci or future PE refinements.

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Cut the nonsense: small molecule modulators of CFTR premature termination codon variants

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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, identified by screening a library of 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 introns 14-17), including mCherry (red) at the N-terminus, eGFP (green) at the C-terminus, and a Flag-tag for detection of plasma membrane–localized CFTR by immunofluorescence with anti-Flag antibody in unpermeabilized cells. NMD inhibition stabilizes the PTC-bearing transcript, increasing mCherry signal, whereas read-through restores full-length CFTR translation, increasing eGFP signal. Two series of novel compounds (a total of 57 compounds) [2, 3] were tested alongside the SMG1i NMD inhibitor and the G418 aminoglycoside read-through compound as experimental positive controls.

Results: Of the 57 novel compounds screened, we chose the seven most promising for further investigation. Toxicity was assessed using the Resazurin assay, and found to be negligible at most experimental concentrations, for six of the seven compounds. We then measured the effects of varying concentrations of the seven compounds on stabilization of CFTR mRNA in gene-edited 16-HBE cells expressing three different PTC variants. Four of the seven compounds produced significantly enhanced CFTR mRNA abundance at certain concentrations alone, and two (s2c11 and s2c12) demonstrated additional activity in combination with SMG1i or G418. We then demonstrated that the combination of s2c11 and SMG1i could enhance protein expression in W1282X cells. Primary cell models (such as intestinal organoids) are being used to validate the potential application of these compounds.

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Integrating reporter mouse lines and mRNA profiling to identify genome editing target cells

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As genome editing strategies evolve, there continues to be a need for platforms that will deliver the editors to relevant cells, such as CFTR-expressing cells and the stem cells that give rise to them. Identifying those cells *in vivo* remains a challenge and is a goal of this study. We have generated mouse models that sensitively detect edited cells in live animals (as few as 10^6 cells in a live animal), and from excised organs (10^4 cells detectable) using luminescence catalyzed by akaluciferase, and single cell detection in tissue sections using fluorescence microscopy of the bright red fluorescent protein, mScarlet. The reporter is a fusion of mScarlet and akaluciferase and can be detected as mRNA as well. Coupled with spatial mRNA profiling and single-cell RNAseq, one can use the reporter system to identify cells at high resolution by gene expression patterns and determine if desired cells have been edited *in vivo* as well as quantify the number and distribution of edited cells.

The reporter system, driven ubiquitously by the CAG promoter and inserted into the *Rosa26* locus, has the reading frame from exons 11 and 12 of human *CFTR* with the R553X premature stop codon, a CF-causing mutation. Translation of the reporter elements occur if the R553X mutation is corrected by editing and a nuclear-localized reporter fusion protein that fluoresces from mScarlet and luminesces via akaluciferase. A prototype editing and delivery system, engineered virus-like particles (eVLPs) carrying an adenine base editor (ABE8) and a gRNA recognizing the R553X sequence are being tested to determine if viral receptor biology can be used to predict particle tropism. The eVLPs efficiently repair R553X in primary cells *in vitro*, purportedly by recognizing the LDL receptor to which the eVLPs' VSV glycoprotein (VSVG) facilitates endocytosis. RNAseq data sets from intestinal tissue, single-cell RNAseq data sets from duodenum and ileum, and spatially profiled mRNA expression of 5,000 genes along the length of the entire small intestines (10X Xenium) were generated. These data provide a rich resource for identifying CFTR-expressing cells and stem cells (*Lgr5*+, for example) and cell surface proteins that could be used to target those cells by future eVLP designs. Single-cell analyses show more than half (10,864/20,045, n=4 mice) of all cells sampled from ileum express *Ldlr*, while 81.5% of 6,821 *Cftr*-expressing cells and 72.2% of 985 *Lgr5*+ cells also express *Ldlr*, making this receptor a good choice for evaluating the approach. Current studies are underway to test the VSVG-containing eVLPs in reporter mice for their tropism for cells expressing LDL receptor and determine if there are any other patterns of gene expression that predict delivery or editing *in vivo*, and how route of administration (systemic or by gavage) influences the overall editing process.

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Out with the old, in with the new? a functional comparison of Vanzacaftor/Tezacaftor/Deutivacaftor and Elexacaftor/Tezacaftor/Ivacaftor in patient-derived intestinal organoids with rare CFTR variants

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Introduction: The introduction of modulator therapies has allowed for a paradigm shift in the overall quality of life in people with cystic fibrosis (pwCF). These modulators act on the cystic fibrosis transmembrane conductance regulator (CFTR) protein by promoting the correct folding and trafficking of the protein to the cell membrane (correctors), and by enhancing the opening probability of the CFTR channel (potentiators). A common triple combination therapy, including two correctors, Elexacaftor (VX-445) and Tezacaftor (VX-661), and a potentiator, Ivacaftor (VX-770), was approved for treatment of pwCF that carry at least one variant with a deletion on the Phenylalanine 508 (F508del). Recently, a novel triple therapy of Vanzacaftor (VX-121)/Tezacaftor/Deutivacaftor (VX-561) (VTD), was approved for more CF genotypes than ETI. In this study, we set out to compare the efficacy of ETI and VTD treatment in a panel of (rare) genotypes in patient-derived organoids (PDIOs). We aim to validate whether VTD treatment is more efficient than ETI, as indicated by *in-vivo* studies.
Materials and Methods: We investigated ETI and VTD treatment using a functional readout with forskolin-induced swelling (FIS) assays. We included common genotypes and a set of rare CFTR mutations. In addition to combinatorial functional rescue, we characterized protein maturation and separate compound potentiation via western blot and FIS assays, respectively.
Results: We show that VTD treatment results in more forskolin-induced swelling and more effective CFTR trafficking than ETI. We compared this effect on a reference panel with homozygous and heterozygous F508del variants, and genotypes in which no CFTR protein is produced (X/X). As expected, neither VTD, nor ETI, rescues CFTR production in X/X genotypes. Additionally, we analysed the separate corrector and potentiator functions of the individual modulators. VX-121 and VX-445 showed potentiator activity, which was synergistically increased by combination with VX-770, indicating a co-potentiative effect. When investigating the effect of both treatments on rare CF genotypes, we saw no significant differences between responders of ETI and VTD.
Conclusion: Overall, we observed a higher relative rescue and functional restoration of CFTR with VTD treatment, compared to ETI. However, we show that there are no significant differences in the response rate of different CF genotypes between ETI and VTD. Thus indicating the possibility of expanding the list of approved CF genotypes for ETI treatment for allowing for a more cost-efficient treatment option.

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FDA-approved drug screen to identify modulators of alternative chloride channel SLC26A9

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Highly effective CFTR modulator therapies led to an unprecedented improvement in lung function in patients with cystic fibrosis (CF) carrying responsive mutations in the *CFTR* gene. However, ~5% of CF patients have no therapy available that targets the basic CF defect. There is an unmet need to develop mutation-independent strategies to bypass CFTR dysfunction and restore ion transport in CF, while such therapy may also be beneficial in other chronic muco-obstructive airway diseases. Evidence suggests that the alternative chloride channel SLC26A9 is a potential candidate target for ion transport modulation.
To identify SLC26A9 modulator drugs, we performed high-throughput screening of the NIH Clinical Collection drug library in CFBE41o- cells with stable expression of SLC26A9 by a live-cell microscopy-based assay using a membrane potential sensitive dye (FLIPR). To validate hit compounds, we investigated the drug effects on SLC26A9-mediated chloride current in Ussing chambers. To study mode of action, we measured *SLC26A9* transcript levels and performed surface biotinylation experiments. For pre-clinical testing, we generated highly differentiated primary nasal epithelial cultures form 12 healthy individuals and 6 patients with CF homozygous to class I mutations, and investigated the drug effect on transepithelial ion transport.
From the high-throughput screening, 11 drug hits were further tested in SLC26A9-overexpressing CFBE41o- cells by electrophysiology. Transepithelial ion transport measurements showed that 24 h treatment with compound 09 led to a significant ~25% increase of the SLC26A9-mediated chloride current.
Treatment with compound 09 did not affect SLC26A9 mRNA expression, whereas surface biotinylation experiments indicated increased amount of protein in the apical membrane by compound 09 compared to vehicle control. Furthermore, testing of compound 09 in healthy nasal epithelia showed a significant ~30% increase in SLC26A9-mediated chloride current, as indicated by the response to specific inhibitor S9-A13. Similarly, compound 09 led to a significant ~30% enhancement of SLC26A9-mediated chloride secretion in CF nasal epithelial cultures compared to vehicle control.
We have identified an FDA-approved SLC26A9 modulator drug by high-throughput screening and validated it in CF patient-derived primary airway epithelial cultures, representing a promising candidate for drug repurposing.

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P 81

Enhancing CF nonsense mutation readthrough via combined small molecule and ACE-tRNA therapies

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Cystic fibrosis (CF) is a genetic condition stemming from mutations in the CFTR gene, which disrupts chloride transport and leads to significant issues in both the respiratory and digestive systems. Roughly 10% of individuals with CF carry nonsense mutations. These mutations create premature stop codons (PTCs) in the mRNA, ultimately resulting in truncated proteins that are typically nonfunctional and rapidly degraded. Current treatment strategies aimed at addressing these mutations focus on enabling readthrough of the PTCs to restore either full or partial CFTR function. This is generally achieved through small molecules that influence the translation termination processes, including compounds like PTC124, various aminoglycosides (such as G418), and release factor degraders like SRI-41315 and CC-90009. Additionally, engineered tRNAs with edited anticodons (ACE-tRNAs) can recognize premature stop codons and insert specific amino acids at the termination site to bypass the nonsense mutation.

While these strategies have shown encouraging results in laboratory settings, their practical application is often hindered by issues like toxicity, low effectiveness, or delivery challenges. This study proposes that a combination therapy, integrating small molecules that modify translation termination with suppressor tRNAs, could potentially boost the effectiveness and/or potency of individual treatments, leading to a reduced delivery burden and more substantial CFTR function restoration in cells with nonsense mutations.

By employing a dual-luminescent reporter system for each stop codon (UAA, UAG, UGA) stably expressed in HEK293 cells, we evaluated the readthrough effectiveness and potency of each small molecule and ACE-tRNA individually. While PTC124 did not show any activity, the other small molecules tested resulted in a dose-dependent suppression of the PTCs. Moreover, the ACE-tRNAs demonstrated greater efficacy than any single small molecule. A dose-response analysis of the ACE-tRNAs was conducted with co-administration of the EC10 for each active small molecule. Although combining the ACE-tRNA with G418 did not enhance activity or potency, the small molecules that depleted release factors, such as eRF1 and its GTPase eRF3, led to increased activity and/or potency. Specifically, CC-90009, which degrades eRF3, enhanced the activity of the leucine-incorporating ACE-tRNA by approximately 1.5 to 2 times compared to the tRNA monotherapy. On the other hand, the eRF1 degrader SRI-41315 not only elevated the activity of the leucine ACE-tRNA by about 2.5 to 3 times but also improved the potency of all studied ACE-tRNAs. In fact, the EC50 values for the ACE-tRNAs were decreased by roughly 50-75% when co-administered with an EC10 of SRI-41315. Currently, we are determining the CFTR functional benefit of combining ACE-tRNAs with these release factor degrader molecules.

These findings support the hypothesis that a combinatorial approach using small molecules to modulate translation termination, coupled with suppressor tRNAs, offers a promising strategy to increase the effectiveness of readthrough therapies for CF patients with PTCs.

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Codelivery of in vitro Transcribed CFTR mRNA with CFTR modulators enhances efficacy of ion transport correction

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Background: In vitro transcribed (IVT) mRNA has emerged in recent years as a promising approach to CFTR protein replacement therapy, but problems remain of limited transfection efficiency in the airway epithelium and short duration of persistence of the transfected CFTR mRNA template. Post transfection, the folding efficiency of endogenous wild-type CFTR protein is low so that even in in non-CF donors, only 20-30% of protein reaches the apical membrane of the epithelium. Inefficient folding of wild-type CFTR from, *in vitro* transcribed (IVT) CFTR mRNA is also, therefore, likely to be a major factor affecting the efficacy of CFTR mRNA therapeutics in the airway. There is, therefore, a window of opportunity to enhance the efficacy of IVT CFTR mRNA therapeutics by improving folding during translation and, thus, translocation to the apical membrane where the protein is more stable. CFTR correctors were developed to enhance folding of CFTR F508Delta but also enhance folding of wild type CFTR.
Aims: The aim of this study was to investigate whether co-delivery of CFTR corrector drugs (VX-809, VX-661 and VX-445) with IVT CFTR mRNA would improve folding of CFTR and so enhance protein stability, translocation to the apical membrane and so improve efficiency of CFTR-mediated ion transport.
Methods: CFTR modulator drugs are hydrophobic and were therefore incorporated into the lipid compartment of the LPNP formulations, co-formulated with the IVT CFTR mRNA for co-delivery of corrector drugs (VX-809, VX-661, VX-445) with the CFTR mRNA encoding the wildtype protein. The resulting CFTR corrector-mRNA LPNPs were characterised for size and charge and transfection efficiency to ensure they retained their transfection efficiency. Transfections were performed in CF basal cells initially and Western blot analysis was performed to assess protein production and proportion of the mature form, band C. Finally, transfections were performed in ALI cultures of CF cells for analysis of CFTR ion transport by Ussing chamber analysis.
Results: We found that codelivery of the CFTR corrector VX-809 with CFTR mRNA in LPNPs enhanced stability of the wild-type CFTR protein encoded by the mRNA by Western blot analysis, with increased proportion of the mature form, band B. VX-661 and VX-445 were less effective. In addition, Ussing analysis showed that ion transport efficiency was enhanced almost to normal levels, while modulator alone and mRNA alone were less effective. The biophysical properties of the LPNP formulation were unaltered by the addition of modulators and there was no effect on mRNA transfection efficiency.
Conclusion: The LPNP formulation allows co-formulation and delivery of CFTR mRNA with VX-809, which significantly improved CFTR expression basal cell transfections. This points to the possible clinical benefit of combined therapies for CF of CFTR mRNA, in particular with the specific corrector VX-809.

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Functionalized nonsense suppressor tRNA picovectors represent a novel therapeutic cargo for the treatment of PTC-associated CF

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Background: 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). For people with CF (pwCF), ~10% harbor CFTR a variant that could be responsive to therapies targeting the PTC codon, for which current CFTR modulators are ineffective. We have recently demonstrated the function of a series of nonsense suppressor anticodon edited tRNAs (ACE-tRNAs) to suppress CF-causing PTCs in the cystic fibrosis transmembrane regulator (*CFTR*) gene in gene-edited immortalized human bronchial epithelial (16HBE14ge) cells. 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 impart several favorable characteristics to the vectors including 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 produce a robust PTC suppression response.

Methods: 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 hairpins serving to covalently close the sLPV ends. Further, these hairpins were synthesized to contain functionalized nucleotides for click chemistry labeling with azide functionalized moieties. Introduction of this chemical functionality allows for dual labeling of the sLPVs with any combination of azide functionalized fluorophores, peptides, proteins, or other DNAs to improve either cellular delivery, intracellular trafficking, or tracking 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. To test the NLS-functionalized sLPV in a manner consistent with delivery to post-mitotic cells, aphidicolin was utilized to arrest cell division in 16HBEge cells.

Results and Conclusions: We tested several NLS sequences, with a scrambled NLS peptide serving as a control. All NLSs improved PTC suppression in aphidicolin treated 16HBEge cells. While the presence of dsDNA in the cytoplasm of human cells can trigger the cGAS-STING innate immune pathway, sLPV labeled with NLS produces ~80% less cGAMP as compared to plasmid DNA. This novel modular sLPV assembly procedure allows for production of minimal ACE-tRNA therapeutic vectors with properties properly tuned for optimal cellular delivery and intracellular trafficking.

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No country for bystanders: a split and inducible precise adenine base editor for the correction of W1282X

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Cystic Fibrosis (CF) nonsense mutations account for roughly 23% of disease-causing variants. Approximately 85% of people with CF who have a nonsense mutation on one allele are compound heterozygous with a modulator responsive variant on the other allele. But that leaves roughly 1 in 7 whose second variant, such as W1282X, does not respond to modulators. W1282X, the fifth most common CF-causing variant, generates a premature terminating codon, causing lack of full-length functional CFTR protein production and nonsense-mediated mRNA decay. Adenine base editing (ABE) can potentially correct any CF-nonsense point mutation with minimal levels of unwanted insertions/deletions. ABE uses a fusion of a Cas9 nickase and a synthetic TadA enzyme modified to convert Adenine (A) into Guanine (G) in an editing window covering varying stretches of nucleotides in the spacer region depending on the deaminase promiscuity. The Harrison lab has previously reported efficient A-to-G editing of W1282X[1], but, high levels of bystander editing at the adjacent downstream A residue, leading to the unwanted generation of the R1283G CF-causing type II variant, directed us to search for alternative strategies.Based on previous reports of reduced off-target/bystander editing and duration of nuclease activity upon splitting one of the TadA domain protein of the base editor complex into two halves[2], we designed two fusion proteins that split the Cas9 nickase: NLS-FKBP12-Cas9(C)-NLS and TadA-Cas9(N)-FRB. In the presence of rapamycin, these should dimerize to form a functional ABE; we refer to these two proteins collectively as splitABE.

The editing window of the parental ABE7.10-SpRY and splitABE was characterized in two different genomic loci with four different gRNAs in HEK293T cells to assess both on-target and bystander editing. Editing was detected across positions 4 to 7 of the spacer region, with the highest editing at position A5. In the presence of rapamycin (72 hours), splitABE showed 53% A-to-G editing levels at position A5, only slightly lower than ABE7.10-SpRY (67%). In the absence of rapamycin, editing at position A5 was only 10% for splitABE indicating a low level of spontaneous dimerization. Overall, this specific 5.1-fold increase in editing confirms that splitABE can be used to regulate editing in a drug-dependent manner, offering the possibility for temporal control of editing.

Future work will focus on assessing the optimal rapamycin concentration and exposure time to achieve precise correction with the least bystander-edits. We will use these conditions to assess if splitABE can edit W1282X without generating bystander-edits in the 16HBE14o-W1282X cell line and the BCi-NS1.1-W1282X immortalized cell line that can differentiate in air-liquid interface culture conditions. This will lead to an understanding of the potential for reduced bystander-edits of splitABE, as well as the impact of the temporal control of editing as a safer gene editing approach.

[1]PMID:37649273

[2]PMID:34663942

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CFTR Gene editing with a super-exon using homology-independent targeted integration – HITI

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Background: Ten percent of individuals with Cystic Fibrosis (CF) lack personalized therapy. The high allelic heterogeneity of the *CFTR* gene makes a mutation-agnostic approach necessary.

Aims: We developed a Super-Exon (SE) gene editing strategy targeting the second half of *CFTR* gene to correct, in a single step, >700 CF-causing variants, while preserving the endogenous promoter. Instead of using HDR for repair of double-strand breaks induced by CRISPR/Cas9 editing system, we employed Homology-Independent targeted Integration (HITI) based on Non-Homologous End Joining end capture, which can target both dividing and non-dividing cells.

Methods: Selection of the sgRNA_C was based on technical development on HEK293T cell lines. We optimized the design of SE $^{11\text{-}27}$ DNA donor sequence to: (1) facilitate the SE$^{11\text{-}27}$ recognition by the spliceosome machinery, (2) stabilize the *CFTR* transcripts derived from edited alleles, and (3) preserve near-native translation while minimizing coding sequence changes. Co-transfection of pSpCas9(BB)-2A-GFP-sgRNA_C and SE11-27-pUCIDT plasmids was performed in W1282X and N1303K 16HBEge cells. Editing efficiency was compared with our previous data using a shorter SE (SE$^{23\text{-}27}$, Mention et al., 2023).

Results: After GFP-positive cell enrichment, SE $^{11\text{-}27}$ based HITI achieved up to 35% SE integration (vs. 5.6% in our previous work) and up to 21.6% of transcripts containing SE$^{11\text{-}27}$ (vs. 7.6%). Preliminary Using Chamber data showed an increase of CFTR chloride channel function that is above the background.

Current work focuses on edited W1282X, N1303K, and F508del clonal cells to further quantify and characterize CFTR transcripts and proteins derived from edited alleles and to assess off-target effects using GUIDE-Seq.

Conclusion: The SE$^{11\text{-}27}$ based HITI strategy is highly efficient and offers promising perspectives for CF therapies.

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Role of miRNAs in CFTR processing in cystic fibrosis: focus on patient response to CFTR modulator therapy

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CFTR modulator therapy has revolutionised cystic fibrosis (CF) care by directly targeting the underlying genetic defect. Potentiators and correctors, alone or in combination, have demonstrated significant improvements in lung function, exacerbation frequency, and nutritional status in individuals with Class II and III CFTR mutations. Despite these advances, variability in patient response remains a major clinical challenge, with mechanisms underlying differential outcomes poorly understood.

To address this, we generated patient-specific induced pluripotent stem cells (iPSCs) from three individuals homozygous for the F508del mutation who exhibited good, moderate, or poor responses to Elexacaftor/Tezacaftor/Ivacaftor (ETI). These iPSCs were differentiated into airway basal cells (iBCs) and profiled for microRNA (miRNA) and gene expression. Dysregulated miRNAs are implicated in CF pathophysiology, influencing CFTR expression as well as pathways related to innate immunity, inflammation, endoplasmic reticulum (ER) stress, and ion conductance. Transcriptomic and miRNA analysis were performed using differential expression and pathway enrichment pipelines

Bioinformatics analysis revealed distinct gene expression signatures between responder groups, particularly in ER stress and PI3K signalling pathways. Furthermore, pathway enrichment analysis revealed significant differences in PI3K pathway activity in poor and moderate responder groups when compared to a good responder. Target prediction analysis of the top expressed miRNAs from a good ETI responder suggests tighter regulation of ER stress components and CFTR processing machinery when compared to a poor responder. A top candidate, miR-405-5p was found to be highly expressed in iBCs derived from a good ETI responder, with validated targets including TP53, HSPA5, and HSPB1 — proteins intricately involved in CFTR processing and cellular stress tolerance. Inhibition of p53 reduces PTEN activation, thereby sustaining PI3K pathway activity, suggesting a mechanistic link between miR-405-5p and ETI responsiveness.

Ongoing experiments will test these hypotheses directly. Transfection of miR-405-5p into poor and moderate responder iBCs will assess whether modulation enhances ETI response, measured by Forskolin-induced swelling assays (FIS). Conversely, inhibition of miR-405-5p in good responder iBCs will be performed using anti-miR oligonucleotides delivered via DNA tetrahedrons. To further probe PI3K pathway involvement, the miR-148-152 family will be transfected to suppress PIK3CA, with pathway activity monitored through pAkt protein expression. CFTR functional rescue across groups will be quantified by FIS assays.

By integrating patient-derived cellular models, miRNA profiling, and pathway analysis, this study aims to uncover molecular determinants of variable ETI response. Results to be presented will provide mechanistic insights into CFTR modulator efficacy and highlight potential biomarkers or therapeutic targets for personalised CF treatment.

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Engineered virus-like particles successfully deliver adenine-base editing to restore CFTR function by correction of R553X

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Premature termination codons (PTCs) in the CFTR gene present a major unmet therapeutic need in cystic fibrosis (CF), as individuals with Class I nonsense mutations such as R553X do not respond to current modulator therapies. Adenine base editing, a CRISPR-based technology that allows the transition of A-T base pairs into G-C base pairs in a targeted manner, offers a strategy to directly and precisely restore sense codons and CFTR expression. A major challenge is delivery of base editors in vivo in a safe and efficient manner. Engineered virus-like particles (eVLPs) offer a transient, non-integrating method for delivering base editor ribonucleoproteins directly to airway epithelial cells.

Vesicular stomatitis virus glycoprotein (VSV-G)-pseudotyped eVLPs were employed to deliver ABE8e with an R553X-specific sgRNA into 16HBEge-R553X cells. Gene editing outcomes were quantified by PCR and next-generation sequencing, and CFTR protein restoration was assessed by Western blot analysis. BE-eVLP transduction achieved dose-dependent correction of R553X, up to ~93% correction of R553X alleles, accompanied by recovery of CFTR protein to ~80% of wild-type expression. Edited cells maintained stable correction over three months, with corrected alleles increasing to ~99% over time, suggesting a proliferative advantage conferred by restored CFTR function.

We evaluated editing in primary nasal epithelial cells from R553X reporter mice harboring an Aka-Luciferase–mScarlet reporter construct containing the human R553X sequence. Highly efficient editing was achieved when cells were transduced with VSV-G-pseudotyped eVLPs, while in suspension, coincident with seeding. With established, confluent and polarised monolayers, basolateral-transduction resulted in many fluorescent and edited cells, but not apical-transduction. Estimates from reporter luminescence indicate basolateral transduction was 81-times more potent at delivering editing than apical.

Functional rescue of CFTR was tested by electrophysiology on 5-week-old polarised monolayers of primary nasal epithelial cells from humanised CFTR $^{R553X/R553X}$ mice. Using chamber assays demonstrated near wild-type levels of CFTR-mediated ion transport when transduction with VSV-G-BE-eVLPs (15 µl) was coincident with seeding onto filters. Short-circuit current measurements post-forskolin-treatment were: 4 µA for untreated verses 78 µA for transduced. Monolayers transduced apically, 2 days post-seeding, with 15 µl VSV-G-BE-eVLPs, registered a short-circuit current of 20 µA after 5 weeks in culture.

Robust and durable repair of the R553X CFTR mutation was achieved with substantial restoration of CFTR function in human and primary airway epithelial cells. VSV-G-pseudotyped eVLPs are most efficient at transducing polarised airway epithelial cells from the basolateral surface.

Given that PTC mutations lack effective treatments, BE-eVLP-delivered base editing represents a promising therapeutic strategy addressing an important unmet clinical need in CF. Future work will address pseudotyping of eVLP platforms to restore therapeutically meaningful CFTR function by targeting specific cell types in the lung and intestine; taking into account delivery route-requirements for polarised cell-surface targeting.

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Gene therapy for Y122X mutation of *CFTR*

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Background: Currently, cystic fibrosis treatment by the potentiator and correctors cocktail ETI (Elexacaftor/Tezacaftor/Ivacaftor) has considerably improved prognosis for almost 90% of patients. However, for mutations that do not respond to ETI no targeted therapies are available.

Aims: In this context we chose to develop a gene therapy strategy based on the prime editing (PE) method derived from the CRISPR/Cas9 technology and its delivery through Virus Like Particles (VLPs).

We selected the Y122X (c.366T>A) mutation as our target, a nonsense mutation located in exon 4 of the *CFTR* (Cystic Fibrosis Transmembrane conductance Regulator) gene which encodes the CFTR channel and that does not respond to ETI.

Methods: We first designed ipegRNAs (intronic prime editing guide RNAs) and associated nicking guides (PE3 strategy), an addition to the prime editing strategy that is known to improve edition. Then, we screened all these constructions by transfection in cell line models HEK293-Y122X (knock in cells generated by lentiviral infection) and 16HBEge-Y122X (gene edited cells from Cystic Fibrosis Foundation).

In parallel, we evaluated VLPs transduction potential with particles containing the mCherry reporter protein and a prime editing system directed against the HEK3 locus, already evaluated in other cellular models. We transduced our cell line models but also wild type human nasal epithelium cells. We monitored mCherry expression by flow cytometry and measured prime editing efficiency at the HEK3 locus by sequencing.

Results: With the PE3 strategy, we were able to detect up to 25% of correction in the HEK293-Y122X cell line.

For the VLP delivery strategy, we observed a high transduction efficiency, detecting mCherry expression in up to 90% of 16HBEge-Y122X cells and achieving almost 30% of edition at the HEK3 locus for the best conditions in primary cells.

Conclusion: We were able to identify the best PE constructions for Y122X editing. Moreover, we established proof of concept that the VLPs are able to efficiently deliver prime editing tools in our models. We are now testing the complete strategy with VLPs containing the PE constructions for Y122X correction.

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Let's not jump to conclusions: split template jumping prime editing of CFTR exon 12 utilizing Cas12a

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Development of nucleic acid-based therapies (NABTs) is critical for people with CF (pwCF) who are ineligible or intolerant to CFTR modulators. Here we describe the use of Template Jumping Prime Editing (TJ-PE), a gene editing technique that can efficiently rewrite DNA sequences of ~200bp using a Prime Editor (PE)[1]. Of the 26 CFTR exons that are ≤200 bp, we chose exon 12 as a model system – a strategy to correct of all of exon 12 accounts for around one-third of pwCF who are unable to take advantage of modulator treatment. Cas9 prime editors require an NGG PAM site; the most suitable pair of spacer regions we identified resulted in a reverse transcription template (RTT) design of 788 bp due to the low GC content of the intronic flanking sequences. Our initial attempt was successful, but inefficient with 0.008% of PCR products showing correction of the exon 12 sequence.

Further analysis of the exon 12 region identified several TTTV sequences suitable for Cas12a editing. Cas12a has been used for PE but has not yet been utilized in TJ-PE[2]. To develop a TJ-PE with Cas12a, we needed five components. (1) Cas12a nicks and creates a 3'-flap recognized by the primer binding site (PBS) within (2) the circular prime editing template RNA (petRNA). The petRNA can recognize the (3) reverse transcriptase (RT) via MS2/M coat protein (MCP) interaction. We designed (4,5) two gRNAs with 20 base crRNA spacer sequences targeting intron 11 on the top strand and intron 12 on the bottom strand.

This design allowed us to reduce the size of the RTT to 188bp.

We first validated the two gRNAs by transfecting into HEK293T cells with Cas12a and observed efficient targeted excision of the exon 12 region. We next transfected HEK293T cells with both gRNAs, Cas12a, the RT, and the petRNA and observed TJ-PE of ~0.11% (~14-fold more efficient than with Cas9), which more than doubled to 0.25% when repeated in the presence of AZD7648 (DNA-PK inhibitor) to limit Non-homologous End Joining (NHEJ).

We have since modified our gRNA design by increasing the crRNA spacer sequence from 20 bp to 23 bp. 23bp gRNAs and Cas12a were transfected into 16HBEWT cells. DNA sequencing was performed to quantify the targeted excision of exon 12 (12%, n=3). Our next steps include transfections of 16HBEge G542X cells and HEK293T cells with all components of TJ-PE and to measure the level of exon 12 correction. If successful, this could be used to correct all exon 12 mutations in CFTR.

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1. PMID: 37291100
2. PMID: 38200119

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Correction of the CFTR mutation G542X in 16HBE cells using conventional HDR templates with reduced target homology in exon 12

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CRISPR-mediated precision genome editing represents a highly promising therapeutic modality for the correction of genetic disorders, including cystic fibrosis (CF). Targeted double-strand breaks generated by Cas9/gRNA complexes can be accurately repaired through homology-directed repair (HDR) utilizing donor DNA templates. Nevertheless, HDR-based approaches are inherently constrained by their limited capacity to facilitate sequence correction beyond a narrow region surrounding the cleavage site. In CF, which are characterized by multiple pathogenic variants, this constraint significantly diminishes the clinical applicability and therapeutic scope of gene editing strategies.
Exon 12 of the *CFTR* gene contains 18 different CF-causing variants. HDR has been used to correct single CF-causing mutations. However, its widespread application is restricted by indel formation and dependence on nearby PAM sites as editing efficiency declines from the gRNA cut site. We hypothesized that high sequence homology between donor and target DNA contributes to reduced efficiency and triggers early collapse of the D-loop[1] and hinders HDR-mediated repair. To test this hypothesis, we designed 3 HDR templates (HDRTs) with varying degrees of homology HDRT-6 or HDRT-18 (mismatch every 18 bp or every 6bp, respectively) and a conventional template that preserves wild-type exon 12 sequence except for PAM site substitution (HDRT-1). Our objective is to evaluate this hypothesis by screening gRNA and donor template capable of efficiently correcting all CF-causing mutation with exon 12.
In our previous data, three CFTR mutant cell lines with variants at the 5', middle and 3' regions of exon 12 were generated to assess these HDRTs. Amplicon sequencing showed that HDRTs with multiple base changes achieved similar editing efficiencies across all mutations (7.5% c.1585-1G>A, 10.3% G542X, 10.3% c.1679+1G>A), whereas conventional HDR templates exhibited reduced efficiency with increasing distance from the gRNA site (8.0% to 1.3%). These findings suggest that reducing donor-target homology can extend the HDR editing window.
To further explore these findings, we screened several gRNAs in intron 11 and 12 regions. H293T cells were treated with different donor template HDRT_mm6 or HDRT_mm18 (silent substitution every 6th and 18th position respectively) and HDRT_PAM with only change in 2 nucleotides in PAM site with length of donor template extended 300-400 bp in intron 12. Amplicon sequencing of HDRT and Cas9/gRNA transfected cells using a gRNA 349 bp downstream of exon 12 revealed that HDRT_PAM showed higher efficiency (7.46%) than HDRT_mm18(1.67%), HDRT_mm6 (0.66%). D-loop extension was higher with HDRT_PAM change> HDRT_mm18>HDRT_mm6. Next, we plan to test gRNAs which are closer to exon 12 (within 200 bp downstream) to assess how gRNA placement affects HDR efficiency. This approach may enable correction of all CF-causing mutations in exon 12.
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Rescue of the CFTR chloride channel with premature termination codons is markedly improved under inflammatory conditions

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In cystic fibrosis (CF), nonsense mutations produce premature termination codons (PTCs) that hamper the correct CFTR protein translation. The resulting truncated forms of the CFTR chloride channel are insensitive to presently available CFTR modulators (correctors, potentiators). Read-through (RT) of the ribosome stalled at the PTC site by small molecules can allow continuation of the protein synthesis. ELX-02, a promising RT agent, completed phase II clinical trial for people with cystic fibrosis (pwCF). Besides RT agents, small molecules acting as eRF3a degraders, such as CC-90009, have been recently described as effective on PTCs. We evaluated the effect of CC-90009 in combination with ELX-02 on CFTR carrying PTCs. Previous works reported that TNFα and IL-17A treatment can enhance rescue of delF508-CFTR variant by CFTR modulators in human bronchial epithelial cells (HBECs). We investigated whether inflammatory stimuli could also improve pharmacological correction of PTCs.
We generated in vitro fully differentiated HBECs from a patient carrying the G542X mutation and we cultured them on porous supports under air-liquid interface (ALI) condition. We treated the cells with or without IL-4 or TNFα/IL-17A for 72 h. In the last 24 h, cells also received drug cocktails containing ELX-02 (200μM) + VX-809 (1μM), with or without CC-90009 (0.1 μM). We evaluated the rescue of CFTR function by short-circuit current (Isc) experiments, and protein and mRNA levels by automated capillary immunodetection and qRT-PCR, respectively. We performed bulk RNA-seq to assess gene expression changes under treatments.
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. Reduced SMG6 and UPF1 protein expression suggest that cytokine may limit nonsense mediated decay (NMD) process. Gene ontology analysis of RNA-seq data confirmed downregulation of NMD pathway.
Inflammatory stimuli significantly improve RT-based correction of G542X-CFTR. Dissection of the underlying mechanism could lead to identification of novel druggable molecular targets to rescue impairment of protein synthesis. Furthermore, pharmacological rescue of PTC-CFTR could be more effective than expected in vivo due to inflammatory conditions.
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CFTR Gene expression, DNA methylation patterns of CFTR locus and the impact of an epigenetic treatment

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Background: Cystic Fibrosis (CF) is caused by pathogenic variants of the CFTR gene. CFTR-expressing cells are heterogeneously committed within the differentiated respiratory epithelium. Most of them are secretory cells, although a considerable amount of CFTR is produced by the rare FOXI1-expressing ionocytes. In addition, several regulatory elements can modulate CFTR gene expression in a tissue-specific manner. Patient-specific cellular models are useful for the comprehension of genetic and epigenetic mechanisms of respiratory epithelium differentiation and their alteration in CF.

Aims: The aim was the study of mRNA expression and DNA methylation patterns of wild-type and mutated (F508del/F508del) CFTR gene. We also tested a CFTR epigenetic amplificatory strategy by DNA hypomethylation of patient-derived cells.

Methods: Using both wild-type and F508del/F508del patient-specific model of nasal epithelial stem cells, inducible to differentiation, we tested the hypomethylating drug 3-deazaadenosine (3-DZA), acting on the S-adenosyl-methionine (SAM) pathway of methyl-donor. The CFTR expression analysis was performed by digital droplet PCR in the following experimental conditions: nasal brushings, conditionally reprogrammed epithelial stem cells, air-liquid interface (ALI)-differentiated cells and ALI-differentiated cells treated with 3-DZA. The DNA methylation analysis was performed in 7 regulatory genomic regions of the CFTR locus, including the -80.1 kb, the -44kb, the -35kb, the -20.9kb, the 5'-flanking, the intron 26 and the +48.9 kb regulatory regions. All CG, CHG and CHH moieties of these regions were studied by bisulfite transformation and next generation sequencing of all experimental conditions.

Results: CFTR gene expression resulted to be enhanced by the DNA hypomethylating treatment, in wild-type and mutated (F508del/F508del) genotypes of ALI-differentiated tissues. A pattern of DNA methylation correlated to CFTR expression levels was evidenced for some CG moieties of the -80.1kb, -35kb, - 20.9kb, 5'-flanking and intron 26 CFTR regulatory regions, including enhancers and CTCF elements, of both ex vivo and in vitro samples. The methylation pattern provides information about the 3-DZA treatment efficacy.

Conclusions: The 3-DZA treatment showed to be able to induce an amplified CFTR gene expression correlated to a DNA hypomethylated pattern of specific CG moieties. These studies evidence the importance of CG sites of regulatory regions of the CFTR locus for the modulation of CFTR gene expression. We propose a model of three-dimensional chromatin organization, involving enhancer and CTCF sites within the transcription activation domain (TAD) of CFTR, modulated by DNA methylation. These findings provide new insights into the role of epigenetics in general, and of DNA methylation in particular, in defining new epigenetic therapeutic strategies in CF.

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The socioeconomic determinants of lung function in children with cystic fibrosis

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Children with cystic fibrosis (CF) from socioeconomically deprived backgrounds are known to have poorer health outcomes, including reduced lung function. This service evaluation aimed to assess the relationship between socioeconomic status and lung function in children with CF at Royal Manchester Children's Hospital (RMCH), and to compare findings with national data.
A retrospective evaluation was conducted using anonymised data from 129 paediatric CF patients. Variables collected included: IMD decile (as a measure of socioeconomic status), percent predicted FEV_1, BMI z-score, sweat chloride, CF genotype, number of antibiotic courses over a period of 12 months, and whether compliance issues were reported. Data were reviewed for normality via histograms. Relationships between FEV_1 and individual variables were assessed using Pearson correlation coefficients. Scatter plots with linear trendlines were generated to visualise the relationship between IMD decile and FEV_1. P values were calculated to assess statistical significance.
A weak positive correlation was found between IMD decile and percent predicted FEV_1 (R^2 = 0.06), indicating that children from less deprived backgrounds had slightly better lung function. This relationship was weaker than national trends, where socioeconomic deprivation explains a larger proportion of lung function variability. Other variables, such as BMI z-score and treatment adherence, also showed associations with FEV_1.
Findings suggest that while socioeconomic disparities in lung function persist, RMCH demonstrates a flatter social gradient than national data. This may reflect effective local clinical practices that help reduce health inequality in children with CF.

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From HTS to patient-specific phenotyping: CBF-based assessment of CFTR modulators in hiPSC-derived airway epithelia

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Although significant progress has been made in CF therapy research over recent decades, there remains a need to improve available treatments, particularly for patients with rare or currently untreatable CFTR mutations. Robust preclinical evaluation of new modulators requires patient-specific airway models that accurately recapitulate the central pathophysiology of CF lung disease —defective mucociliary clearance (MCC) arising from impaired epithelial ion transport and dehydration of the airway surface liquid - and that can be produced at scale. Human induced pluripotent stem cell (hiPSC)–derived airway epithelia address this need by enabling the production of ciliated air–liquid interface cultures from individual patients. However, cellular inhomogeneity and variable maturation in hiPSC-derived epithelia can compromise epithelial uniformity and complicate conventional CFTR function assays (e.g., Ussing chamber short-circuit current, halide flux, membrane potential assays) highlighting the need for complementar, scalable, automatable readouts that capture key determinants of MCC. Ciliary beat frequency (CBF), which integrates ciliary motility with airway surface liquid properties meets this criteria. Combined with hiPSC-derived airway epithelium, CBF enables patient-specific phenotyping and provides a practical platform for CF drug discovery and modulator assessment. This framework also supports theratyping by quantifying genotype-specific responses to CFTR-modulators in patient-derived cultures, to inform the use of approved therapies for rare mutations.

We aim to establish an automatable, mid- to high-throughput CBF assay as a screening-oriented platform for CFTR-modulators using hiPSC-derived airway epithelium that models impaired MCC, with modulator-induced rescue of CBF as the primary readout. In addition, we assess the feasibility of using this iPSC-based workflow to evaluate CFTR-active compounds identified in a previous iPSC-based high-throughput screening (HTS) study and refined through medicinal chemistry.

The CBF assay provided a stable, automatable platform that reflected the CF lung disease phenotype in hiPSC-derived airway epithelium. Treatment with established CFTR-modulators partially restored CBF, consistent with clinical observations of incomplete functional restoration and confirming phenotypic rescue. The assay’s sensitivity to different modulator combinations supports its suitability for compound ranking. Standardizing the workflow (file-based aquisistion, scriped analysis) demonstrated feasibility for unattended operation and reduced hands-on analysis time. Preliminary results suggest that small-scale, iterative testing of HTS-derived, medicinal chemistry-optimized CFTR active compounds can be conducted within the same iPSC/CBF framework.

In summary, the CBF assay delivers stable measurements in hiPSC-derived airway epithelia and could serve as a scalable, patient-specific, automatable platform for assessing CFTR-modulators and supporting theratyping across CFTR genotypes. While full high-throughput deployment will require further automation and formal performance benchmarking, current data support the use of the CBF assay for the phenotypic evaluation of approved modulators and the testing and prioritization of carefully scoped, HTS-derived chemistry-refined candidates with the ultimate goal of expanding therapeutic options for patients with rare CFTR mutations.

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Proof of in-vivo sphingolipid Delta-4 desaturase (DEGS) reversible inhibition in mice pups following administration of ETI to dams during pregnancy and brestfeeding

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We already demonstrated (1,2) that Tezacaftor inhibits the enzyme (DEGS) which converts dihydroceramides (dHCer) into ceramides (3), thus producing increase of dHCer in various cells and tissues. Since DEGS malfunctioning is often associated with severe neurological impairments linked with aberrant myelin composition and structure (4,5), we conducted an in vivo drug safety study, by administering Elexacaftor/Tezacaftor/Ivacaftor (ETI) to CD-1 mice during pregnancy and breastfeeding. The aim of this work is to evaluate the potential impact of DEGS inhibition by ETI on the formation of the peripheral and central nervous systems (PNS, CNS). ETI was incorporated as powder into mouse food, in a high-fat diet regimen. Besides recording weight and size, we also investigated pups' behavior by means of dedicated tests. ETI and dHCer levels in plasma and tissues, as well as changes in the global lipidome were measured by tandem mass spectrometry coupled to liquid chromatography. At 10 days after birth, we observed a significant accumulation of dHCer in the brains of pups born from ETI-fed dams compared to controls. No accumulation was observed in the sciatic nerve of these animals, likely due to much lower levels of ETI in this tissue compared to the brain. We also conducted an untargeted lipidomics survey, which revealed other alterations in lipid metabolism associated with exposure to ETI during pregnancy. During breastfeeding, when exposure to the drug decreases, these alterations revert and virtually disappear 28 days after birth, together with other differences in the phenotype and behaviour of the pups observed earlier during development (6). We also conducted a longer-term study, by administering ETI to mice from pregnancy to adulthood (3 months) and we carried out a set of behavioural and molecular investigations. During the project, we faced some experimental difficulties, mostly related to ETI formulation in food, which we are overcoming using also a bit of creativity. Our project is now progressing toward a better understanding of the practical implications of our findings.

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The PD-1 / SHP2 immune checkpoint controls bacterial killing by human CF airway neutrophils

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Objectives: People with CF (pwCF) suffer from chronic airway infections in spite of the massive presence of neutrophil phagocytes in the lung lumen. This paradox is explained by our discovery of an acquired, active tolerance of bacteria by neutrophils recruited to the CF airway milieu (Margaroli, Cell Rep Med 2021). Here, we tested whether this tolerance was controlled, at least in part, by the PD-1 / SHP 2 axis, a well-known immune checkpoint signaling pathway in cancer and autoimmune diseases.

Methods: PD-1 expression was assessed in primary blood and sputum neutrophils from pwCF by flow cytometry. In addition, CF airway supernatant (CF ASN, obtained from sputum, per Dobosh, STAR Prot 2021) was used to transmigrate and condition human blood neutrophils to adopt a bacteria tolerant phenotype. Next, CFASN-conditioned neutrophils were assessed for activation of the SHP2 phosphatase downstream on PD-1. Rescue of their bactericidal activity against *P. aeruginosa* (CF pathogen) was attempted by treatment with an anti-PD1 antibody combined with a SHP2 inhibitor.

Results: PD-1 and its major ligand PD-L1 are highly upregulated on sputum compared to blood neutrophils collected from pwCF. At the transcriptional levels, human blood neutrophils conditioned into CF airway neutrophils in our biomimetic model show a steady increase in SHP2 expression over the course of 2 to 14 hours of transmigration and exposure to CFASN. Remarkably, blockade of the PD-1 / SHP2 axis in these CF airway-conditioned neutrophils restores killing of *P. aeruginosa* to the level observed in control airway neutrophils.

Conclusion: The PD-1 / SHP2 axis is involved in the inhibition of bacterial killing and resulting bacterial tolerance by neutrophils recruited to the CF airway lumen. PDL-1 on neutrophils is the likely ligand for activation of this checkpoint, as we previously showed for PD-1 ligation on macrophages in infants with CF (Margaroli, J Cyst Fibros 2022). Our data support further exploration of PD-1 pathway inhibitors, repurposed from other domains of medicine, as a mutation-agnostic immunotherapy in CF.

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Primary nasal epithelial cells for personalized medicine in cystic fibrosis patients with rare mutations

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Background: Elexacaftor/tezacaftor/ivacaftor (ETI) was initially developed to improve *F508del*-CFTR function in people with cystic fibrosis. Recently, ETI was approved for people with CF with at least one non-class I mutation. However, the efficacy of many rare mutations is unknown. Therefore, we tested the pharmacological rescue of CFTR activity by ETI *in vitro* and *in vivo* in non-*F508del* people with CF to improve understanding of mutation-specific CFTR modulator responses and to support personalized treatment.

Methods: Highly differentiated primary nasal epithelial cells (pHNEC) were established from nasal swabs of 54 people with CF and non- *F508del* mutations as well as 29 healthy controls. CFTR activity was assessed by short-circuit currents in Ussing chamber after ETI or DMSO incubation and expressed as % of healthy CFTR function ($\Delta I_{ETI/DMSO}$%WT). In 12 people with CF, who received ETI therapy after a positive response in pHNEC, we assessed lung function (FEV1% predicted) and the *in vivo* CFTR biomarkers sweat chloride concentration (SCC), intestinal current measurements (ICM) and nasal potential difference (NPD) at baseline and 1 to 3 months after initiation of ETI.

Results: 37 people with CF showed no increased CFTR activity in pHNEC after ETI treatment compared to DMSO. However, 17 people with CF and non-*F508del* mutations showed ETI response with a mean correction of CFTR activity of 20% of the healthy level. 12 people with CF who received ETI therapy showed a mean improvement in FEV_1% predicted by 12%. All 12 patients showed a pathological SCC above 60 mmol/L at baseline, which was reduced by - 36 mmol/L after initiation of ETI ($p < 0.001$). In the ICM, the cAMP-dependent chloride secretory response increased from -6 µA/cm^2 at baseline to 98 µA/cm^2 after initiation of ETI ($p < 0.001$) and the total chloride secretory response increased from -18 µA/cm^2 to 130 µA/cm^2 ($p < 0.001$). A subgroup of 7 patients, where NPD was performed, showed an increase in total chloride response from 2.8 mV at baseline to -12 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 SCC, 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 for people with CF.

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Frameworks to support preclinical development of antibiotic and anti-inflammatory drugs in cystic fibrosis

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Chronic bacterial respiratory infections, particularly those sustained by *Pseudomonas aeruginosa* in cystic fibrosis (CF) and bronchiectasis, continue to impose a major burden on patients and healthcare systems. Despite considerable progress in drug discovery, many candidate antimicrobial and anti-inflammatory therapies fail during clinical development, revealing a critical gap between preclinical efficacy testing and clinical outcomes. Bridging this disconnect requires preclinical platforms that better reflect the complexity of chronic lung disease and incorporate endpoints aligned with patient response. We established and validated a comprehensive translational platform combining lipopolysaccharide (LPS) exposure, acute and chronic mouse lung infection models with multi-parameter readouts that monitor both pathogen colonization and host immune response. Chronic infection was induced using agar-embedded *P. aeruginosa*, enabling prolonged bacterial persistence and faithfully representing hallmarks of CF advanced disease, including biofilm formation, mucus obstruction, neutrophil-dominated inflammation sustained by IL-17A, and progressive tissue remodeling.

To determine the experimental conditions that most accurately predict therapeutic responses, we evaluated benchmark antimicrobial (Cigana et al. Eur Respir J. 2020) and anti-inflammatory (Cigana et al. Biomed. Pharmacother. 2025) compounds under different administration routes and treatment timings. Chronic infection models, especially when early treated, provided the most clinically relevant scenario for detecting anti-inflammatory and anti-bacterial efficacy, with observable improvements in inflammatory markers and overall health status. Acute infection models, while valuable for assessing antibiotic activity, were poorly suited to capture anti-inflammatory effects and occasionally worsened airway inflammation. Likewise, models based on lipopolysaccharide-driven pulmonary inflammation did not adequately reproduce therapeutic responses seen in chronic infection. Among the evaluated readouts, those directly linked to pulmonary pathology, such as bronchoalveolar lavage leukocyte counts, neutrophil elastase, myeloperoxidase and cytokine or chemokine profiles in lung tissue, proved superior to systemic hematological parameters in sensitivity and translational relevance. During chronic infection, effective control of airway inflammation was frequently followed by reduced bacterial burden, highlighting the interdependence between host-directed therapies and pathogen clearance.

This validated framework is implemented within Cystic Fibrosis animal Core Facility (CFaCore), supported by the Italian Cystic Fibrosis Research Foundation (FFC), which provides researchers with scientific, technical and regulatory expertise to advance the development of antibacterial or anti-inflammatory therapies. CFaCore has already supported the preclinical progress of novel compounds, including GY971, an investigational anti-inflammatory drug candidate that showed significant efficacy in our platform and is currently advancing through FFC's preclinical “Derisking” pipeline to support its further development. Overall, our work reinforces the need for harmonized and disease-relevant preclinical strategies that align model selection, therapeutic context and biologically meaningful endpoints. By offering a scalable and predictive framework for drug validation, our platform contributes to accelerating the development of effective therapies for CF and to improving the likelihood of clinical success.

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A novel scalable assay to quantify CFTR function in human epithelial organoids

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The forskolin-induced swelling (FIS) assay estimates CFTR function by measuring swelling of intestinal organoids. It has advanced personalized CF treatment and drug screening substantially in the last decade. The assay demonstrates that patient-derived material can be used to test drugs for specific CF genotypes on a group level. However, the FIS assay fails to measure the full spectrum of CFTR functionality due to its two-dimensional nature and relative units of measurement. This results in poor estimations in inconclusive genotypes and unreliable drug screenings if restored function is limited. We hypothesize that measuring swelling in 3D and normalizing for the number of cells in an organoid results in more sensitive measurements that are absolute and comparable across experiments. We aim to develop a new functional CFTR assay that appreciates complex 3D structures and is measured in absolute values to accurately measure CFTR function across the full functional spectrum.
To address these limitations, we present the average surface area per nucleus (ASAP-N) measurement in organoids via live nuclear stains and confocal microscopy. Normalizing organoid size by the number of cells enables the direct comparison of different organoids. To obtain this absolute value, we developed an AI-based analysis pipeline that segments nuclei with automated quality control and estimates the 3D surface area of an organoid. This automated pipeline enables robust, high-throughput quantification of organoid morphology.
This 3D imaging of the nuclei of organoids shows a limited difference in ASAP-N between wild-type (WT) and F508del/F508del organoids prior to CFTR activation (WT = 120.4 μm^2/cell vs F508del = 105.1 μm^2/cell; $\sigma = 0.149$). However, after forskolin stimulation, a period of swelling is observed that stops after approximately 2 h, keeping the organoids in a steady state for at least the next 14 h. In this time period, the ASAP-N of individual organoids can be measured. We found that forskolin-stimulated WT intestinal organoids exhibit a significantly larger ASAP-N (227.5 μm^2/nucleus) than stimulated F508del organoids (95.9 μm^2/nucleus) ($\sigma < 0.001$).
These absolute values of minimal and maximal functional CFTR genotypes observed in patient-derived intestinal organoids provide us with the boundaries of the functional CFTR spectrum. In the future, we aim to use the ASAP-N assay for known residual-function and inconclusive CF genotypes to see whether the full functional spectrum can be observed.

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Systemic effects of cystic fibrosis transmembrane conductance regulator (CFTR) modulators on the plasma and serum proteome

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Cystic fibrosis (CF) is caused by a dysfunction in the cystic fibrosis transmembrane conductance regulator (CFTR) and affects multiple organs by obstructing mucus and altering secretion. While the combination drug elexacaftor/tezacaftor/ivacaftor (ELX/TEZ/IVA, ETI) has markedly improved clinical symptoms, its broader molecular and systemic effects remain to be fully elucidated.

Using mass spectrometry-based proteomics, we compared the plasma and serum proteomes of people with cystic fibrosis (pwCF) who were treated with the earlier, less effective lumacaftor/ivacaftor (LUM/IVA) combination with those who received the more potent ELX/TEZ/IVA therapy. Our analysis revealed specific and common pharmacodynamic signatures associated with inflammation and metabolic processes under each treatment regimen. Notably, the ELX/TEZ/IVA therapy produced more consistent alterations in pwCF that resembled profiles observed in healthy individuals.

Furthermore, by comparing the sputum and serum proteomes of patients with cystic fibrosis (pwCF) treated with ELX/TEZ/IVA, we identified counter-directional changes in pulmonary surfactant-associated protein B (SFTPB), a potential biomarker of lung permeability. These changes correlated with improvements in lung function and could be validated in an independent cohort.

This study provides a comprehensive resource that enhances our understanding of CFTR modulator-driven proteome alterations. It offers insights into systemic and local protein regulation in cystic fibrosis (CF). Our findings suggest that ELX/TEZ/IVA promotes broader systemic health improvements and provide critical information that could inform future CF therapies.