2023

European Cystic Fibrosis Society

18th ECFS Basic Science Conference

Conference Programme & Abstract Book

Dubrovnik - Croatia

Chairpersons

Nicoletta Pedemonte

Alexandre Hinzpeter and Camille Ehre

29 March – 01 April 2023

To have access to all of the abstracts, please download the full abstract book.

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CONFERENCE SPONSORSHIP & SUPPORT

The ECFS thanks the following for their support

WELCOME FROM THE ECFS PRESIDENT

It is a great pleasure to welcome you in Croatia to the 18th European Cystic Fibrosis Conference dedicated to Basic Science.

This year we are delighted to welcome Dr. Nicoletta Pedemonte as the conference Chairperson who will be supported by Dr. Alexandre Hinzpeter and Dr. Camille Ehre as co-chairpersons.

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

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

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

A very warm welcome to an exciting conference.

Isabelle Fajac
President
European Cystic Fibrosis Society

WELCOME FROM THE CONFERENCE CHAIRPERSONS

A very warm welcome to you to the 18th ECFS Basic Science Conference located, this year, in Croatia.

It's easy to see why Dubrovnik is known as the, "Pearl of the Adriatic" as soon as one arrives. By taking a walk around the historic centre, outstanding views can be seen from the famous city walls encircling the city. Built to protect Dubrovnik from invaders in days gone by, history seeps out from the stones, allowing tourists to step back in time and discover the long, rich history. In the past, Dubrovnik was known for fruitful merchandising and maritime trading. Nowadays it is one of the most popular tourist destinations in the Adriatic region.

A main feature of the Basic Science Conference is the large presence of young participants, including PhD students and post-docs, who are instrumental to the success of this annual event with their passion and participation in lively discussions.

During the week there will be ample opportunity to share and explore your data with others and interact in this beautiful setting with the best of European and International experts within this field.

This year's programme features 8 symposia with invited speakers together with invited talks from submitted abstracts. There are also two keynote lectures and flash poster sessions. This conference will be a unique chance to discuss topical aspects of basic research in cystic fibrosis with international experts.

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

Nicoletta Pedemonte
Istituto Giannina Gaslini, Genoa
Italy

Alexandre Hinzpeter
INSERM U1151, Institut Necker Enfants Malades, Paris
France

Camille Ehre
University of North Carolina, Chapel Hill
United States

18th ECFS Basic Science Conference

29 March – 01 April 2023, Dubrovnik, Croatia

Programme

Nicoletta Pedemonte (Genoa, IT), Alexandre Hinzpeter (Paris, FR),
Camille Ehre (Chapel Hill, US)

Wednesday, 29 March 2023 (Day 1)	
19:00-19:30	**Official Opening of the Conference by the Conference Chairpersons**
19:30-20:15	**Welcome Reception**
20:15-21:30	*Dinner*

Thursday, 30 March 2023 (Day 2)	
07:30-08:45	*Breakfast*
08:45-09:45	**Opening Keynote Lecture**
	Single-channel studies of CFTR - David Sheppard (UK)
09:45 -10:15	*Coffee break & Poster viewing*
10:15-12:00	**Symposium 1 – CFTR structure and function**
	Chairs: Alexander Hinzpeter (FR) - Cédric Govaerts (BE)
10:15-10:40	Regulation of the CFTR chloride channel through its interaction with protein kinase A - László Csanády (HU)
10:40-11:05	Regulation of CFTR function by post-translational modifications - Mauro Salvi (IT)
11:05-11:30	F508del-CFTR rescue by second-site mutations in its transmembrane domains - Paola Vergani (UK)
11:30-11:40	Abstract 04 - A synonymous polymorphism associated with rare CFTR variants confers alterations to protein biogenesis, pharmacologic response, and clinical phenotype - Kathryn Oliver (US)
11:40-11:50	Abstract 01 - Extending the success of Trikafta to rare mutations - insights from CFTR structure and modulator binding - Noemie Stanleigh (IL)
11:50-12:00	Abstract 11 - Exploring the potentiating effects of VX-445 on CFTR: a functional perspective - Maria-Cristina Ardelean (UK)
12:00-13:00	**Flash Poster Session (even numbers)**
	Chair: Olivier Tabary (FR)
13:00-14:15	*Lunch*

14:30-16:15	**Symposium 2 – CFTR interactome**
	Chairs: Mauro Salvi (IT) - Carla Ribeiro (US)
14:30-14:55	An overview on CFTR interactions: old partners and new players - Carlos M. Farinha (PT)
14:55-15:20	CFTR dysfunction and its impact on exocrine/endocrine signaling in the CF ferret pancreas – John Engelhardt (US)
15:20-15:45	Pharmacological chaperones, which rescue CFTR-F508del, target a gatekeeper controlling cargo access to ER exit sites - Stefano Marullo (FR)
15:45-15:55	Abstract 15 - A PI3Kγ mimetic peptide promotes F508del-CFTR localization at plasma membrane through a PKD1-dependent mechanism - Marco Mergiotti (IT)
15:55-16:05	Abstract 17 - Effect of the lipid environment on the F508del CFTR rescue and stability at the plasma membrane - Nicoletta Loberto (IT)
16:05-16:15	Abstract 18 - Non-canonical inhibitors target CAL binding: new approaches to stabilize functional F508del-CFTR - Dean Madden (US)
16:15-16:45	*Coffee break* & **Poster viewing**
16:45-18:30	**Symposium 3 – Care for rare: precision medicine to bring innovative therapies to people with rare mutations (a patient organisation-initiated symposium)**
	Chairs: Nicoletta Pedemonte (IT) – Elise Lammertyn (BE)
16:45-16:53	Patient view
16:53-17:22	Learning from rarity: lessons from studies on disease-relevant models, in the path from basic defects to personalized therapies – Nicoletta Pedemonte (IT)
17:22-17:51	*Ex vivo* models / translational models - Isabelle Sermet Gaudelus (FR)
17:51-18:20	Regulatory aspects, hurdles and needs on the way to realise personalised medicine in cystic fibrosis - Marco Cipolli (IT)
18:20-18:30	Abstract 57 - Pan-ethnic characterization of CFTR variants - Justin Ideozu (US)
20:00-21:30	*Dinner*
21:30-23:00	**Evening Poster Session:** Posters with Even numbers

Friday, 31 March 2023 (Day 3)	
07:30-08:45	*Breakfast*
08:45-10:30	**Symposium 4 – Mucus and mucins**
	Chairs: Camille Ehre (US) - Giulio Cabrini (IT)
08:45-09:10	Mucin in high resolution - Deborah Fass (IL)
09:10-09:35	Progressive development of abnormal mucus precedes chronic *P.Aeruginosa* infection in the cystic fibrosis rat - Susan Birket (US)
09:35-10:00	The distinct components of airway mucus – Anna Ermund (SE)
10:00-10:10	Abstract 71 - Low molecular weight alginate oligosaccharides enhance the diffusion of lipid-nanoparticles through cystic fibrosis mucus - Ruhina Maeshima (UK)
10:10-10:20	Abstract 21 - Structure of domains in polymerizing mucins and their importance for the formation of normal and CF mucus - Gunnar C Hansson (SE)
10:20-10:30	Abstract 23 - Sputum viscoelasticity is driven by concentration in organic components - Lydia Esteban Enjuto (FR)

10:30-11:00	*Coffee break* & **Poster viewing**
11:00-12:45	**Symposium 5 – Inflammation and modulators**
	Chairs: Deborah Fass (IL) - Luis Galietta (IT)
11:00-11:25	The CF airway inflammatory milieu enhances the efficacy of CFTR modulators – Carla Ribeiro (US)
11:25-11:50	Molecular targets for anti-Inflammatory therapies - Giulio Cabrini (IT)
11:50-12:15	Inflammation, resolution and repair in the era of highly effective modulator therapy for CF - Robert Gray (UK)
12:15-12:25	Abstract 36 - Pulmonary ketogenesis promotes tolerance to *P. Aeruginosa* infection in the CF lung - Sebastian Riquelme (US)
12:25-12:35	Abstract 16 - Differential neutrophilic responses to *Pseudomonas aeruginosa* reveal novel protein-based treatments relevant to Cystic Fibrosis - Tia Rizakos (CA)
12:35-12:45	Abstract 38 - Fertility deficiency in female cystic fibrosis patients: determining the role of the uterus' endometrium using cutting-edge organoid models - Ellen De Pauw (BE)
12:45-14:00	*Lunch*
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18:30-19:30	**Flash Poster Session (odd numbers)**
	Chair: Susan Birket (US)
20:00-21:30	*Dinner*
21:30-23:00	**Evening Poster Session:** Posters with Odd numbers

Saturday, 01 April 2023 (Day 4)	
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09:10-09:35	Understanding conformational transitions favoring CFTR NBD1 unfolding to design conformation-specific therapeutics – Cédric Govaerts (BE)
09:35-10:00	Design of novel potentiators based on protein-protein interactions - Norbert Odolczyk (PL)
10:00-10:10	Abstract 46 - A novel CFTR corrector displaying a unique mode of action - Peter van der Sluijs (NL)
10:10-10:20	Abstract 50 - Use of computer simulations to investigate ivacaftor-induced conformational changes in CFTR - Diana-Florentina Veselu (UK)
10:20-10:30	Abstract 54 - Elexacaftor/Tezacaftor/Ivacaftor triple therapy corrects function of CFTR rare variants - Isabelle Sermet Gaudelus (FR)
10:30-11:00	*Coffee break* & **Poster viewing**

11:00-12:45	**Symposium 7 – Gene-based molecular approaches**
	Chairs: Marie Egan (US) - Elise Lammertyn (BE)
11:00-11:25	New approach to treat all CF patients with an antisense oligonucleotide - Olivier Tabary (FR)
11:25-11:50	RNA-based therapies for cystic fibrosis - Stephen Hart (UK)
11:50-12:15	Mutation-tailored base and prime editing approaches allow re-writing CFTR to treat cystic fibrosis. – Marianne Carlon (BE)
12:15-12:25	Abstract 58 - A precise adenine base editor corrects W1282X-CFTR without bystander effects - Lúcia Santos (PT)
12:25-12:35	Abstract 59 - Targeted repair of cystic fibrosis mutations across an entire exon using Cas9 and a single guide RNA and donor template - Emma Collins (IE)
12:35-12:45	Abstract 75 - CFTR functional rescue after delivery of nebulized LNP/mRNA to primary human bronchial epithelial cells derived from patients with different CFTR genotypes - Daniella Ishimaru (US)
12:45-14:00	*Lunch*
14:15-16:00	**Symposium 8 – Alternative targets for cystic fibrosis**
	Chairs: Carlos M. Farinha (PT) - Isabelle Sermet Gaudelus (FR)
14:15-14:40	Pharmacological therapies for PTC mutations - Luis Galietta (IT)
14:40-15:05	The chloride versus bicarbonate dilemma in airway muco-obstruction. Lessons from the basolateral side - Carlos Flores (CL)
15:05-15:30	Development of an hiPSC-based CF lung disease model to study TMEM16A as an alternative therapeutic target in cystic fibrosis – Sylvia Merkert (DE)
15:30-15:40	Abstract 76 - Identification of pharmacological modulators of the calcium-signaling cascade - Michele Genovese (IT)
15:40-15:50	Abstract 82 - TAVT-135, a novel chloride ion transporter for the pan-genotypic treatment of cystic fibrosis: electrophysiological and mucus-hydration properties - Roger Ilagan (US) – Presenter Andreas Maetzel (US)
15:50-16:00	Abstract 78 - BK modulation as a therapeutic target for Cystic Fibrosis - Arthur Mitchell (UK)
16:00-16:30	*Coffee Break*
16:30-17:30	**Closing Keynote lecture**
	In vivo gene editing - Marie Egan (US)
20:00	*Dinner / Social Event*

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P75 CFTR functional rescue after delivery of nebulized LNP/mRNA to primary human bronchial epithelial cells derived from patients with different CFTR genotypes
Daniella Ishimaru, Ishita Agarwal, Ali Alfaifi, Rumpa Bhattacharjee, Dmitri Boudko, Sofia Chavez, Sierra Comini, Emmanuel Fasusi, Mirko Hennig, Arunan Kaliyaperumal, Lucy Kipyator, David Liston, Ella Meleshkevitch, Sakya Mohapatra, Touhidul Molla, Omid Mousa, Maninder Sidhu, Berto Tejera-Hernandez, Christine Tran, Philip Thomas, Vladimir Kharitonov, Brandon Wustman, David Lockhart

P76 Identification of pharmacological modulators of the calcium-signaling cascade
Michele Genovese, Martina Buccirossi, Daniela Guidone, Fabio Bertozzi, Tiziano Bandiera, Luis J. V. Galietta

P77 SLC26A9 and ATP12A as potential therapeutic targets for cystic fibrosis
Giulia Gorrieri, Floriana Guida, Ilaria Musante, Rosaria Casciaro, Fabiana Ciciriello, Federico Zara, Paolo Scudieri

P78 BK modulation as a therapeutic target for cystic fibrosis
Arthur Mitchell, David Benton, Guy Moss, Vivek Dua

P79 Identification of predictive biomarkers from the graft associated with the development of primary graft dysfunction after lung transplantation in CF patients
Damien Adam, Caroline Landry, Ariane Jalbert, Mays Merjaneh, Nicolas Noiseux, Basil Nasir, Ahmed Menaouar, Charles Poirier, Emmanuel Charbonney, Pasquale Ferraro, Emmanuelle Brochiero

P81 Identification of novel NMD modulators to rescue nonsense mutations in cystic fibrosis
Arianna Venturini, Anna Borrelli, Isabelle Sermet-Gaudelus, Luis Juan Vicente Galietta

P82 TAVT-135, a novel chloride ion transporter for the pan-genotypic treatment of cystic fibrosis: electrophysiological and mucus-hydration properties
Roger Ilagan, Martina Gentzsch, József Maléth, Nancy L. Quinney, Viktória Szabó, Florina Zákány, Orsolya Basa-Dénes, László Molnár, Istvan M. Mandity

P83 Gene-targeting approach to investigate the role of TMEM16A (ANO1) in airway epithelia
Martina Buccirossi, Michele Genovese, Daniela Guidone, Fabrizio Andreone, Jlenia Monfregola, Luis J. V. Galietta

P84 Targeting the oxidative imbalance for CFTR rescue in cystic fibrosis
Ilaria Artusi, Michela Rubin, Angela Menna, Anna Pianazzola, Valentina Bosello-Travain, Giorgio Cozza

P86 Characterization and activation of adenosine receptor pathway in CFTR function
Paula Barranco-Bartolomé, Laia Grau Romero, Silvia Gartner, Julio Cesar Castro-Palomino, Elena García-Arumí, Eduardo F. Tizzano

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Feng Liang, Haibo Shang, Yi Chen, Jan Harrington, Stephanie Cantu, Lan Wang, Jennifer Shepard, Kevin Coote, Hermann Bihler, Martin Mense

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Anita Guequen, Bárbara Tapia, Sandra Villanueva, Carlos Flores

P90 Effect of Tristetraprolin overexpression in CF
Solenne Bleuse, Jessica Varilh, Karine Deletang, Marion Nadaud, Prisca Boisguerin, Arnaud Bourdin, Magali Taulan-Cadars

P91 Fluorescence-based measurements of CFTR channel activity in primary nasal epithelial cultures recapitulates Ussing chamber measurements
Tarini Gunawardena, Zoltan Bozoky, Claire Bartlett, Hong Ouyang, Theo Moraes, Tanja Gonska, Christine Bear

P92 An upstream airway-specific enhancer contributes to SLC6A14 modification of lung phenotypes in CF
Mohsen Esmaeili, Naim Panjwani, Cheng Wang, Fan Lin, Adele Chan, Gengming He, Katherine Keenan, Julie Avolio, Ann Harris, Johanna Rommens, Lisa Strug

P94 Antisense oligonucleotide targeting TMEM16A: a mutation agnostic therapy for cystic fibrosis
Christie Mitri, Nathalie Rousselet, Harriet Corvol, Olivier Tabary

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Tarini Gunawardena
Tia Rizakos

30 March — 08:45–09:45

Opening Keynote Lecture

Single-channel studies of CFTR

David N. Sheppard

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

Single-channel recording provides the opportunity to observe in real time the behaviour of the cystic fibrosis transmembrane conductance regulator (CFTR), the ATP-binding cassette transporter, which functions as an epithelial anion channel. In combination with other techniques to quantify CFTR function at the tissue level and evaluate the biosynthesis of CFTR protein and its plasma membrane stability, single-channel recording helps to provide molecular explanations for CFTR dysfunction in cystic fibrosis (CF) and the action of small molecule CFTR modulators.

In this presentation, recent studies of the impact of CF-causing CFTR variants on the single-channel behaviour of CFTR will be discussed. For example, in nucleotide-binding domain 1 (NBD1), the highly conserved residue S549 is the site of three CF-associated CFTR variants: S549R, S549N and S549I. At constant cellular mRNA levels, the amount of mature S549N-CFTR protein resembled that of wild-type, whereas those of S549R- and S549I-CFTR protein were markedly reduced. In combination, the CFTR corrector lumacaftor and the CFTR potentiator ivacaftor restored greatest CFTR-mediated transepithelial Cl^- current to the S549-CFTR variants with the magnitude of S549N-CFTR function being equivalent to that of wild-type. In excised inside-out membrane patches from transiently transfected CHO cells studied at 37 °C, the S549 variants were without effect on current amplitude and thermostability, but altered channel gating. By reducing the apparent ATP affinity and efficacy, S549N- and S549R-CFTR impeded greatly channel gating. By contrast, S549I-CFTR slowed channel opening and closing without altering the open probability of CFTR. Except for S1347R-CFTR, the same variants at the equivalent residue in NBD2 (S1347) were without effect on channel gating. Thus, S549 plays a key role in CFTR processing and gating.

In membrane-spanning domain 2 (MSD2), serine 1159 in the pore-lining twelfth transmembrane segment is the site of two rare CFTR variants S1159F and S1159P. When studied in excised inside-out membrane patches from transiently transfected CHO cells incubated at low temperature to enhance channel trafficking to the plasma membrane, S1159F- and S1159P-CFTR formed Cl^- channels activated by cAMP-dependent phosphorylation and gated by ATP that exhibited themostability at 37 °C. Both CFTR variants modestly reduced the single-channel conductance of CFTR. By severely reducing the apparent affinity and efficacy of CFTR for ATP, S1159F- and S1159P-CFTR strongly attenuated the frequency and duration of channel openings, resulting in marked reductions of open probability. Ivacaftor (10 -- 100 nM) doubled the open probability of both CFTR variants, but did not restore their open probability to wild-type levels. Thus, S1159F and S1159P are gating variants, which also affect CFTR processing and conduction. These studies of CFTR variants at S549 and S1159 highlight the power of single-channel recording combined with other techniques to elucidate how CFTR variants cause CFTR dysfunction to inform the treatment of people with CF using CFTR-targeted therapeutics.

I thank past and present laboratory colleagues for their commitment and dedication, generous collaborators for the opportunity to work together to investigate CFTR variants and the CF Trust and CF Foundation Therapeutics for sustained support for single-channel studies of CFTR.

30 March — 10:15–12:00

Symposium 1 – CFTR structure and function

S1.1 Regulation of the CFTR chloride channel through its interaction with protein kinase A

Mihályi, C[1,2,3], Iordanov, I.[1,2,3], Csanády L.[1,2,3]

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Gating of the CFTR anion channel pore is regulated by ATP binding and hydrolysis at its two cytosolic nucleotide binding domains (NBDs) and phosphorylation of its regulatory (R) domain at multiple serines by cAMP-dependent protein kinase (PKA). The pore opens upon formation of a tight NBD dimer following ATP binding, and closes upon disruption of that dimer following ATP hydrolysis. Because in live cells CFTR's NBDs are continuously saturated with ATP, physiological regulation of CFTR channel activity is mediated by the catalytic subunit of PKA (PKA-C) which is released from the holoenzyme in response to signaling events that increase cytosolic cAMP concentration. Despite its prime physiological relevance, the molecular mechanism of CFTR activation by PKA-C is poorly understood. The R domain is an unstructured peptide segment. In cryo-EM structures of unphosphorylated CFTR it is wedged between the two NBDs, preventing their dimerization, whereas in structures of phosphorylated CFTR it is released from this inhibitory position (Liu et al.2017, Cell 169: 85-95; Zhang et al., 2018, PNAS 115:12757-62). Functional studies suggest an equilibrium between "wedged-in" and "released" R-domain conformations which is biased towards wedged-in for the unphosphorylated R domain, but can be shifted towards released not only by phosphorylation, but also through simple binding of PKA-C, causing additive irreversible and reversible CFTR activation (Mihályi et al., 2020, PNAS 117:21740-6). In recent studies we have started to address the structural determinants of the CFTR/PKA-C interaction. Using competition experiments we demonstrate that reversible CFTR activation requires a direct interaction between the R domain and the PKA-C substrate binding site. Furthermore, we find that the efficiency of PKA-C towards CFTR is isoform-specific: compared to the native bovine enzyme (bPKA-C), bacterially expressed recombinant PKA-C (rPKA-C) is less efficient to phosphorylate CFTR and fails to support reversible activation. Differences in posttranslational modification patterns between the two isoforms include autophosphorylation at serine 10 in rPKA-C, but not bPKA-C, and N-myristoylation in bPKA-C, but not rPKA-C. Selective manipulations of these posttranslational modifications in both isozymes point towards a key role of PKA-C N-myristoylation for efficient CFTR activation. The potential relevance of these findings for CF lung disease will be discussed.

S1.2 Regulation of CFTR by post-translational modifications

Christian Borgo[1], Claudio D'Amore[1], Luca Cesaro[1], Valentina Bosello Travai[2], Mauro Salvi[1]

[1]Department of Biomedical Sciences, University of Padova, Italy., [2]Department of Molecular Medicine, University of Padova, Italy.

The CFTR protein undergoes several post-translational modifications (PTMs), including phosphorylation, ubiquitination, sumoylation, and methylation. The possibility of targeting the enzymes that regulate these PTMs has been the subject of intensive research over the years, aimed at identifying possible pharmacological approaches to correct or compensate for pathogenic mutations. Indeed, regulation of PTMs could influence the stability of the protein, control trafficking to the plasma membrane, and regulate channel gating properties. Several serine/threonine and tyrosine phosphorylation sites have been identified. The most important phosphorylation sites, mainly located within the regulatory R domain, are those generated by the protein kinase PKA and are required for CFTR opening. Another kinase responsible for the phosphorylation of the channel is the ubiquitous protein kinase CK2. However contrasting results have been produced over the years regarding the role of this kinase. If in the past CK2 inhibition, has been proposed as a promising therapeutic intervention for treating misfolded mutants recent evidence suggested that CK2 activity is necessary for proper channel function, although the target sites of CK2 regulating CFTR are still elusive. Here we discuss some new findings from our lab on the role of CK2 on CFTR.

Direct inhibition of protein ubiquitination for years has been considered a promising therapeutic approach for the treatment of misfolded mutants that undergo premature polyubiquitination and degradation, but despite a lot of scientific efforts, no specific compound has reached the clinic. Only recently we and others have demonstrated that to be effective, this approach must be addressed at the beginning of the ubiquitination cascade, and, in combination with correctors, it leads to an improvement in CFTR conductance on cells expressing F508del and other rare CF-causing mutations. Protein ubiquitination is also regulated by crosstalk with other PTMs. Based on the observation that in CFTR some lysines can undergo both ubiquitination and methylation, we recently hypothesized that preventing deubiquitination could be an effective strategy to block ubiquitination and channel degradation. Our research led to the identification of KDM2A and KDM3B as potential targets to increase the stability of the F508del protein, however, we failed to demonstrate a direct role of methylation on channel function. Again, the crosstalk between sumoylation and ubiquitination has been previously described as an important mechanism to regulate channel stability. Some new results by our group about the role of sumoylation on channel stability will be here presented.

S1.3 How *in cis* revertant mutations in ICL4 can rescue F508del-CFTR

Stella Prins[1], Valentina Corradi[2], David N. Sheppard[3], D. Peter Tieleman[2], Paola Vergani[1]

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CFTR is the anion-channel mutated in cystic fibrosis (CF). The most common CF-causing mutation is deletion of F508 (F508del). In wild-type (WT) CFTR, F508, at the interface between nucleotide binding domain 1 (NBD1) and intracellular loop 4 (ICL4), plays an important role by linking domains. ICL4 extends between transmembrane helix 10 (TM10) and TM11, forming a "domain-swapped" element within transmembrane domain 2 (TMD2). ICL4, together with ICL1 (between TM2 and TM3, in TMD1), tether NBD1 to the TMDs. This "ball-and-socket" joint is known to be important, for CFTR folding/stability and for relaying conformational signals from the NBDs to the channel gate. This was confirmed by the recent discovery of binding sites for VX-661 and VX-445 (two of the components of the triple-combination CF therapy Trikafta), suggesting that their synergistic action might be related to simultaneous stabilization of ICL1 and ICL4, respectively (1).

We carried out a systematic mutagenesis scan of ICL4, with the aim of identifying second-site mutations capable of reverting the biogenesis and/or functional defects of F508del-CFTR. In the F508del-CFTR background the native residue at each ICL4 position (1064 - 1074) was replaced by F, H, M, Q, W or Y. The resulting panel of 61 CFTR double mutants was screened exploiting an image-based high-content assay which allows simultaneous characterization of CFTR ion-channel function and membrane proximity (2).

The screen results confirmed that the R1070W mutation (3) is a powerful revertant, significantly improving membrane proximity and increasing F508del-CFTR conductance to 42% of the value measured for WT CFTR. In contrast, the R1070F mutation had a minor effect on conductance and no effect on biogenesis of F508del-CFTR. Extensive *in silico* simulations allowed us to compare the molecular dynamics of F508del-R1070F- and F508del-R1070W-CFTR and how these differed from WT- and F508del-CFTR. Both in WT- and in F508del-R1070W-CFTR, aromatic side-chains (F1068, F508 or 1070W, F1074) maintained a loose stack at the NBD1-ICL4 interface. This was distorted in F508del-CFTR but also present in F508del-R1070F-CFTR (F1068, 1070F, F1074). In the latter, however, the NBD1/ICL4 interface had lost the flexibility that characterized the other variants. Both R1070 (in WT and F508del-CFTR) and 1070W made frequent and transient hydrogen bonds with residues within ICL4. Thus, the inability of 1070F to rescue the F508del defects could stem from not allowing transient molecular contacts with hydrophilic partners.

F1068M and F1074M followed R1070W as the most effective revertant mutations, rescuing conductance to 17% and 14% of the value obtained with WT-CFTR. Methionines at these positions could dynamically switch between hydrophilic (4,5) and relatively strong and flexible hydrophobic contacts at the NBD1-ICL4 interface.

Crosslinking NBD1 to ICL4 has been shown to abolish gating (6). In addition, recent results suggest an uncoupling of NBD1 from the network of interactions with ICL1/ICL4 might occur physiologically and modulate gating in WT-CFTR, but trigger misfolding in F508del-CFTR (7,8). The R1070W, F1068M and F1074M revertant mutations, by providing both hydrophobic and hydrophilic NBD1-ICL4 contacts, might allow dynamic sampling of alternate CFTR conformations and provide the required balance between stability and flexibility.

1. Fiedorczuk, K., and Chen, J. (2022) Molecular structures reveal synergistic rescue of Δ508 CFTR by Trikafta modulators. *Science* **378**, 284-290 2. Prins, S., Langron, E., Hastings, C., Hill, E. J., Stefan, A. C., Griffin, L. D., and Vergani, P. (2020) Fluorescence assay for simultaneous quantification of CFTR ion-channel function and plasma membrane proximity. *J Biol Chem* **295**, 16529-16544 3. Thibodeau, P. H., Richardson, J. M., Wang, W., Millen, L., Watson, J., Mendoza, J. L., Du, K., Fischman, S., Senderowitz, H., Lukacs, G. L., Kirk, K., and Thomas, P. J. (2010) The Cystic Fibrosis-causing Mutation deltaF508 Affects Multiple Steps in Cystic Fibrosis Transmembrane Conductance Regulator Biogenesis. *J Biol Chem* **285**, 35825-35835 4. Mundlapati, V. R., Ghosh, S., Bhattacherjee, A., Tiwari, P., and Biswal, H. S. (2015) Critical Assessment of the Strength of Hydrogen Bonds between the Sulfur Atom of Methionine/Cysteine and Backbone Amides in Proteins. *The Journal of Physical Chemistry Letters* **6**, 1385-1389 5. Merski, M., Skrzeczkowski, J., Roth, J. K., and Górna, M. W. (2020) A Geometric Definition of Short to Medium Range Hydrogen-Mediated Interactions in Proteins. *Molecules* **25**

6. Serohijos, A. W. R., Hegedus, T., Aleksandrov, A. A., He, L., Cui, L., Dokholyan, N. V., and Riordan, J. R. (2008) Phenylalanine-508 mediates a cytoplasmic-membrane domain contact in the CFTR 3D structure crucial to assembly and channel function. *Proc. Natl. Acad. Sci. U S A* **105**, 3256-3261 7. Sigoillot, M., Overtus, M., Grodecka, M., Scholl, D., Garcia-Pino, A., Laeremans, T., He, L., Pardon, E., Hildebrandt, E., Urbatsch, I., Steyaert, J., Riordan, J. R., and Govaerts, C. (2019) Domain-interface dynamics of CFTR revealed by stabilizing nanobodies. *Nature Communications* **10**, 2636 8. Scholl, D., Sigoillot, M., Overtus, M., Martinez, R. C., Martens, C., Wang, Y., Pardon, E., Laeremans, T., Garcia-Pino, A., Steyaert, J., Sheppard, D. N., Hendrix, J., and Govaerts, C. (2021) A topological switch in CFTR modulates channel activity and sensitivity to unfolding. *Nat Chem Biol* **17**, 989-997

P4

S1.4 A synonymous polymorphism associated with rare *CFTR* variants confers alterations to protein biogenesis, pharmacologic response, and clinical phenotype

Giovana B. Bampi[1], Robert Rauscher[1], Disha Joshi[2,3], JaNise J. Jackson[2,3], Eric J. Sorscher[2,3], Zoya Ignatova[1], Kathryn E. Oliver[2,3]

[1]University of Hamburg, Department of Chemistry, Institute of Biochemistry and Molecular Biology, Hamburg, DE, [2]Emory University School of Medicine, Department of Pediatrics, Atlanta, US, [3]Emory University and Children's Healthcare of Atlanta, Center for Cystic Fibrosis and Airways Disease Research, Atlanta, Georgia, US

People with CF carry more than 250 different synonymous or “silent” single nucleotide polymorphisms (sSNPs) in *CFTR* that are often viewed as neutral for protein folding and function. We previously identified a relatively common sSNP (c.2562T>G) that inverts local translational speed at the affected codon (T854T), leading to alterations in CFTR topology and ion transport through a mechanism dependent on ribosome velocity. When c.2562T>G is present in cis, this sSNP induces subtle structural rearrangements to counteract destabilizing effects of certain rare *CFTR* variants (G551D, D579G, D614G), thereby enhancing channel function.

In the present study, we assessed impact of the c.2562T>G sSNP on CFTR biogenesis and pharmacologic sensitivity using Fischer rat thyroid cells and CF bronchial epithelia transiently expressing mutant CFTR cDNA with or without c.2562T>G. Our findings indicate this sSNP increases maturation and transepithelial ion transport mediated by rare *CFTR* variants, such as D579G and D614G, following treatment with clinically approved small molecules (e.g. VX-809, VX-770). Additionally, sSNPs engineered to alter the speed of translation at the primary disease-causing mutation (i.e. “revertants”) were found to exert strong effects on CFTR modulator-dependent rescue.

Using whole-genome sequencing data from the CF Genome Project, we next interrogated prevalence and clinical relevance of the c.2562T>G sSNP across 5,058 CF subjects in the United States. Approximately 22% of this population harbors one or two copies of c.2562T>G. This sSNP is predominantly associated with rare *CFTR* variants, as evidenced by a frequency of 0.28% among F508del alleles. Interestingly, patients with the *CFTR* genotype, 3849+10kbC>T / F508del, exhibit substantially different sweat chloride levels depending on the presence or absence of c.2562T>G, which is presumed to reside on the 3849+10kbC>T allele. For these individuals, mean sweat chloride is significantly lower among people who encode c.2562T>G (52.2 mmol/L; SD ± 15.4) compared to those who do not have the sSNP (69.7 mmol/L; SD ± 16.8) ($P = 0.0344$ Mann-Whitney, ranksum).

Our results argue against neutrality of *CFTR* sSNPs during protein biogenesis, highlighting ways in which silent mutations can change local kinetics of mRNA translation and epistatically modulate outcomes of CF-causing variants. Such effects are likely to influence the spectrum of disease symptoms, represent a mechanistic contributor to genotype-phenotype relationships, and ultimately may help predict therapeutic response in precision theratyping studies.

This work is supported by the U.S. National Institutes of Health, U.S. Cystic Fibrosis Foundation, German Cystic Fibrosis Foundation – muko E.V., and Deutsche Forschungsgemeinschaft. In addition, we thank Karen Raraigh and Prof. Dr. Garry Cutting (Johns Hopkins University; Baltimore, Maryland, USA) for providing data from the CF Genome Project.

P1

S1.5 Extending the success of Trikafta to rare mutations - insights from CFTR structure and modulator binding

Noemie Stanleigh[1], Michal Gur[2,3], Eitan Kerem[4], Michael Wilschanski[4], Batsheva Kerem[1]

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Efficient genotype specific CFTR modulators, ELX/TEZ/IVA, are approved, offering therapeutic opportunities for most CF patients. But they have not been approved for many rare mutations. The use of ex-vivo models, such as rectal organoids, enables testing CFTR modulators in individuals not included in clinical trials.

We established patient-derived organoids to test for modulator response of the rare mutation Q1100P. This mutation is not reported in CFTR2, although there are scattered mentions in the literature of patients carrying this allele. We obtained biopsies from 3 patients heterozygous for the mutation. Their second allele was either a stop mutation (C225X that is not expected to be responsive to ELX/TEZ/IVA) or an unreported missense mutation (K193E). The basal CFTR activity as measured by forskolin swelling assay (FIS) was minimal. We assessed function after treatment with each modulator separately or in combinations.

In Q1100P/C225X organoids patient, VX-661 alone had no effect. CFTR function was partially restored with VX-445 and was further increased in combination with VX-661, without additional benefit from VX-770. The Q1100P mutation causes the introduction of a proline residue instead of the WT glutamine, expected to disrupt the alpha-helix structure of TM11. A recently published structure of CFTR in complex with VX-445 (1) shows binding of the modulator to two amino acids flanking Q1100P (1098 and 1102). This raises the possibility that VX-445 binding corrects the structural defect introduced by the Q1100P mutation, leading to functional correction of the protein. VX-661 further increased the correction of CFTR function by VX-445 in these organoids, consistent with other studies showing a synergistic effect of VX-661 and VX-445 in improving the CFTR F508del folding and function (1).

In Q1100P/K163E organoids, CFTR function was partially restored by treatment with VX-661 alone, and significantly restored by treatment with VX-445 alone. Interestingly, no additive effect above that of VX-445 was achieved with the combination of VX-661+VX-445. No additional benefit was seen from addition of VX-770. In these organoids, function restoration might arise from both alleles. K163E is a missense mutation in a conserved lysine residue participating in ICL1-NBD1 interface, which is crucial for CFTR folding and corrected by VX-661 (2). Interestingly, VX-661 in these organoids did not contribute to the high response to VX-445. Further studies with organoids from a patient homozygous for K163E are ongoing and will shed light on the molecular basis of the modulator responses.

Based on our results, treatment with Trikafta was approved by the health provider for two Q1100P/K163E patients. Improvement in clinical parameters was immediate. FEV1 improved by 15% in one patient and 21% in the other, accompanied by a decrease in LCI (by 5.6 and 5 points) and decreased sweat chloride (9 and 30 mmol/L) in the first and second patients, respectively.

Altogether, we show how knowledge of the CFTR structure and modulator binding sites can guide in prioritizing the analysis of rare mutations to existing treatments in patient-derived organoids.

References:

1. Fiedorczuk K, Chen J., Science 2022;378.

2. Kleizen, B. et al., J. Mol. Biol 2021.

P11

S1.6 Exploring the potentiating effects of VX-445 on CFTR: a functional perspective

Maria-Cristina Ardelean, Paola Vergani

University College London, Neuroscience, Physiology and Pharmacology, London, GB

Cystic fibrosis is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene, which encodes an anion channel regulating transepithelial fluid movement. The most prevalent mutation, F508del, impairs folding, leading to reduced CFTR at the cell membrane and defective gating.

The standard-of-care treatment for patients carrying F508del, Trikafta (Vertex Pharmaceuticals), combines the potentiator VX-770 (ivacaftor), the corrector VX-661 (tezacaftor), and the dual-activity modulator VX-445 (elexacaftor). Initially, it was believed that VX-445 operates primarily as a corrector by stabilising CFTR's nucleotide-binding domain 1 (NBD1) (Veit et al., 2020; JCI Insight 5:e139983). However, recent studies demonstrated that it also increases CFTR open probability (Veit et al., 2021; J Cyst Fibros 20:895-898). Despite recent cryo-EM structures of F508del-CFTR bound to VX-445 (Fiedorczuk and Chen, 2022; Science 378: 284–290), the underlying mechanism of action remains to be fully understood. VX-445 also potentiates G551D- and G1244E-CFTR, variants that have mutations which affect ATP-dependent conformational changes associated with gating. In addition, its effects show synergy with VX-770 on G551D-CFTR (Veit et al., 2021; J Cyst Fibros 20:895-898).

We have found that the R1030A mutation, located in transmembrane helix 10, leads to a reduction in the potency of BPO-27, an ATP-site targeting CFTR inhibitor. R1030A also attenuated the severity of the gating impairment caused by the G551D, G1244E, S1251N, and G1349D mutations, positioned at the interface between CFTR's two NBDs. These findings are consistent with R1030A enabling non-canonical open conformations along with NBD-dimerised canonical ones. We aim to investigate the mechanism of action of VX-445 by evaluating its effects on G551D-CFTR and comparing wild-type and R1030A backgrounds.

To conduct our study, we employed a high-content image-based assay that enables the simultaneous quantification of CFTR membrane proximity and ion-channel function at the single-cell level in human embryonic kidney (HEK-293) cells. We utilised the pIRES2-mCherry-YFPCFTR plasmid to co-express CFTR, tagged at its N-terminus with the halide-sensitive yellow fluorescent protein (YFP, H148Q/I152L) and the red cytosolic fluorescent protein mCherry. The latter allows us to determine the areas and borders of cells and their membrane-proximal zones. Through image analysis, we were able to quantify the total CFTR present across cells and the mature, membrane-proximal CFTR based on the position and intensity of YFP-CFTR fluorescence. All constructs were activated via forskolin-mediated phosphorylation in the presence or absence of acutely added VX-445. Once the steady-state was reached, CFTR activity was determined by adding extracellular iodide and analysing the quenching of YFP-CFTR due to iodide/chloride exchange.

Our measurements revealed an enhanced VX-445-mediated potentiation in G551D in the R1030A-CFTR background, suggesting a synergy between the effects of VX-445 and of the R1030A mutation. The underlying mechanism may be similar to the one responsible for the synergistic co-potentiation between VX-445 and VX-770, as some evidence suggests that VX-770, like R1030A, enables non-canonical CFTR openings (Prins et al., 2020; J. Biol. Chem. 295:16529–16544). Further studies are planned to assess whether the R1030A mutation affects VX-770 activation of G551D-CFTR. These experiments could provide valuable insights into the mechanism of potentiation of VX-445.

30 March — 14:30–16:15

Symposium 2 – CFTR interactome

S2.1 An overview on CFTR interactions: old partners and new players

Carlos M Farinha

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

Remarkable progress in CFTR research led to therapeutic development of modulators that rescue the basic defect in some of the most common cystic fibrosis-causing mutations. However, there is still a significant proportion of individuals with CF that lack a causal therapy to rescue the basic defect caused by their specific genotype. Studying CF disease mechanisms by comprehensively understanding CFTR interactions remains key.

The total amount and function of CFTR in the cell depends on numerous interactions that work as molecular switches to modulate folding, endoplasmic reticulum exit, trafficking through the secretory pathway, channel activity and plasma membrane stability.

As CFTR exits the ribosome, its folding is assessed by molecular chaperones and ER-associated lectins that distinguishes between folded and misfolded versions, targeting the later to degradation. If folding is successful, CFTR exits the ER and traffics to the Golgi where its progress is associated with the maturation of its glycan moieties.

At the trans Golgi cisternae, CFTR is targeted to the plasma membrane (PM) where it anchors to the cytoskeleton through a network of protein adaptors, that contribute to bring together (in the right space and time) many other proteins -- including other transporters, channels and trafficking machinery components (Rab GTPases, SNAREs, PDZ-domain-containing proteins) as well as different types of molecular switches (such as kinases and phosphatases). At the PM, these interactions finely tune CFTR function and stability. CFTR mutations affect one or (frequently) several of these processes.

An overview of our current understanding on CFTR interactions will be given, focusing on the relevance of mutation-specific interactions, on the impact of modulating CFTR membrane stability to boost its correction and on how global gene/protein expression patterns can help to identify novel therapeutic avenues.

S2.2 CFTR dysfunction and its impact on exocrine/endocrine signaling in the CF ferret pancreas.

John F. Engelhardt

University of Iowa

Programme note: the details are not authorised for inclusion

S2.3 Pharmacological chaperones, which rescue CFTR-F508del, target a gatekeeper controlling cargo access to ER exit sites

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Partially characterized retention mechanisms involving arginine-based motifs, inhibit Cystic Fibrosis (CF) Transmembrane Conductance Regulator (CFTR) exit from the endoplamic reticulum (ER). The mutagenesis of these RXR motifs partly rescued the transport to the cell surface of the CFTR-F508del mutant found in CF patients.

The GB1 subunit of the metabotropic $GABA_B$-receptor is fully retained in the ER in the absence of the GB2 subunit. GB1 retention depends on a di-leucin and a RXR motif, which are masked when GB1 hetero-dimerizes with GB2, allowing the release of a competent $GABA_B$-receptor heterodimer to the cell surface. PRAF2, an ER gatekeeper, which recognizes these motifs, was shown to play a major role in GB1 subunit retention.

We found that PRAF2 can also interact on a stoichiometric basis with di-leucin and RXR motifs present in the NBD1 domain of both wild type and mutant CFTR, preventing their access to ER exit sites. Overexpression of PRAF2 inhibits cell surface expression of wild type CFTR in a concentration-dependent manner. Because of its lower abundance, compared to wild type CFTR, CFTR-F508del recruitment into COPII vesicles is suppressed by endogenous ER-resident PRAF2. Interestingly, some of the new pharmacological chaperones that efficiently rescue CFTR-F508del loss of function in CF patients, target CFTR-F508del retention by PRAF2 operating with various mechanisms.

These findings open new therapeutic perspectives for rare diseases caused by the impaired cell surface trafficking of misfolded transporters or receptors.

P15

S2.4 A PI3Kγ mimetic peptide promotes F508del-CFTR localization at plasma membrane through a PKD1-dependent mechanism

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Background: The most frequent mutation in cystic fibrosis (CF) patients (F508del-CFTR) results in a trafficking defect of the channel, that is retained in the endoplasmic reticulum (ER) and consequently degraded by activation of the ERAD pathway. Despite currently approved CFTR correctors have revolutionized the CF therapeutic landscape, these molecules displayed suboptimal efficacy in rescuing the trafficking of F508del-CFTR, underlying the need for additional approaches to enhance F508del-CFTR localization at the plasma membrane (PM).

Aims: Since cAMP is known to promote PM CFTR stabilization (*Lobo et al., J Cell Sci, 2016*), we hypothesized that the cAMP-elevating peptide we previously developed (PI3Kγ MP; Patent n° WO/2016/103176; *Ghigo et al., Sci Transl Med, 2022*) could be exploited to enhance F508del-CFTR PM density and increase the therapeutic effect of CFTR correctors.

Methods: Cell surface protein biotinylation and immunogold electron microscopy were used to evaluate CFTR PM density in HEK293T, 16HBE14o- and CFBE41o- cell lines upon PI3Kγ MP treatment. Phosphoproteomics was exploited to unravel potential targets activated by PI3Kγ MP.

Results: We confirmed our initial hypothesis, showing that PI3Kγ MP increased both wt- and F508del-CFTR PM density. However, we intriguingly observed that the effect on F508del-CFTR trafficking was independent on the ability of PI3Kγ MP to boost cAMP in the cell, suggesting an alternative underlying mechanism(s). An unbiased approach based on phosphoproteomics revealed protein kinase D1 (PKD1), a well-established orchestrator of protein trafficking, as being strongly activated by PI3Kγ MP. Interestingly, PI3Kγ MP failed to promote F508del-CFTR stabilization at the PM after PKD1 inhibition, thus revealing PKD1 as the key mediator of the effect of PI3Kγ MP. Finally, PI3Kγ MP synergized with approved cocktail of CFTR modulators Trikafta® (ETI) in increasing F508del-CFTR PM density, indicating that our peptide could be used to enhance ETI therapeutic effect.

Conclusion: This study identifies PI3Kγ and PKD1 as pivotal regulators of CFTR stability at the PM and suggests exploiting the ability of PI3Kγ MP to increase F508del-CFTR PM trafficking and ETI therapeutic effect.

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

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S2.5 Effect of the lipid environment on the F508del CFTR rescue and stability at the plasma membrane

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The CFTR channel exerts its activity at the plasma membrane (PM) level within specific microdomains whose lipid pattern results altered in bronchial epithelial cells derived from patients with cystic fibrosis (CF).

Interestingly, we found that in CF bronchial epithelial cells carrying F508del CFTR the partial restoration of the CFTR lipid environment by administration of the ganglioside GM1 improves the stability of the mutated channel rescued by the treatment with Orkambi[(1)].

The new triple combination of CFTR modulators, Kaftrio, shows a higher efficacy in the rescue of F508del CFTR even if the infection with *Pseudomonas aeruginosa (P.a.)* determines an important destabilizing effect of the rescued mutated channel[(2)].

By analysing the sphingolipid pattern of CF bronchial epithelial cells overexpressing F508del CFTR and treated with Kaftrio, we observed a partial increase in the content of certain gangliosides, such as GM1 and GD1a, that could be, in part, responsible for the higher efficacy of the triple combination. These changes appear to be mainly due to a promotion in the activity of the sialyl-transferase and to the inhibition of the sialidase, two key enzymes involved in the ganglioside metabolism. Conversely, the levels of cholesterol, another lipid normally enriched in lipid domains and promoting the function of CFTR [(3,4)], were found to be decreased in cells treated with Kaftrio.

Based on this evidence, we investigated whether restoring the lipid composition of the CFTR microdomain by administration of GM1 and cholesterol could ameliorate the effectiveness of Kaftrio on rescuing F508del CFTR, also in presence of *P.a.* infection. For this reason, we fed CF bronchial epithelial cells overexpressing F508del CFTR, treated with Kaftrio in presence or not of *P.a.*, either with different molecular species of GM1 or with human lipoproteins with the aim to deliver cholesterol to the cells.

As a result of these experiments, we observed that GM1 administration improves the effect of Kaftrio in term of increased levels of the rescued F508del CFTR at the PM level, and by using different molecular species of GM1, we demonstrated that those carrying shorter acyl chains result to be more effective. Moreover, the GM1 administration partially counteracts the CFTR instability induced by *P.a.* infection.

On the other hand, we found that the treatment of cells with lipoproteins, particularly LDL (low-density lipoprotein), determines a relevant increase in the CFTR levels.

Based on these data, the administration of GM1 and/or cholesterol, together with correctors and potentiators, could be considered as an innovative strategy to ameliorate the effectiveness of CFTR modulators and could also open a new scenario for the treatment of CF patients with orphan mutations affecting folding and stability of the channel.

1. Mancini G, Loberto N, Olioso D, *et al*. Int J Mol Sci (2020)

2. Stanton BA, Coutermarsh B, Barnaby R, *et al*. PLoS ONE (2015)

3. Abu-Arish A, Pandzic E, Goepp J, *et al.* Biophys J (2015)

4. Chin S, Ramjeesingh M, Hung M, *et al.* Cells (2019)

P18

S2.6 Non-canonical inhibitors target CAL binding: new approaches to stabilize functional F508del-CFTR

Dean Madden[1], Nicholas Gill[1], Jeanine Amacher[1,2], Patrick Cushing[1], Yu Zhao[1], Luke Wallace[3], Alex Pletnev[3], Mark Spaller[3], Sierra Cullati[4], Scott Gerber[4], Prisca Boisguérin[5], Dominick Casalena[6], Douglas Auld[6], Siyu Wang[7], Bruce Donald[7]

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The era of highly effective modulatory therapies (HEMTs) requires a comprehensive reassessment of therapeutic strategies for people with CF. While HEMTs have provided substantial improvements in lung function and reductions in pulmonary exacerbations requiring hospitalization, responses vary widely among individuals, and there is clear room for continued improvement and personalized optimization of combination therapies. Such improvements might also address the persistence of chronic airway infections in many patients.

Our prior work has established that endogenous trafficking proteins can serve as therapeutic targets that complement current drug combinations, which consist of corrector and potentiator modulators that address the processing and gating defects of F508del-CFTR, respectively. While highly effective for many patients, approved therapies do not directly address the reduced plasma membrane stability of rescued F508del-CFTR. Thus, we aim to identify drugs (stabilizers) that increase the plasma membrane half-life of CFTR by targeting the CFTR-Associated Ligand (CAL) PDZ domain, an essential mediator of CFTR lysosomal degradation.

Protein-protein interactions are notoriously difficult drug targets. The surfaces are distributed and relatively featureless, forcing trade-offs between potency and specificity, and thus complicating efforts to develop inhibitors. Here, we report a combination of screening, structure-based design, computational modeling, and biochemical and cellular characterization to develop peptide and small-molecule inhibitors of the CAL PDZ domain, as a basis for further development. Our best CAL PDZ peptide inhibitors exhibit single-micromolar affinities for the target binding site. However, they also interact with off-target PDZ domains, some of which may have countervailing negative effects on CFTR stability. We have identified a highly specific variant, but is 10x less potent. To improve affinity without losing specificity, we have now modeled and synthesized non-natural amino acids to reach outside of the canonical peptide binding site, contacting PDZ loops that are more variable, and thus potentially more selective. We report here proof-of-principle using a novel adducted lysine side chain that enhances peptide affinity by forming a stereochemically specific interaction with a distal loop. Our second strategy has been to screen libraries of drug-like small molecules, using a combination of fluorescence-based high-throughput assays. Candidates were selected for further study based on follow-up dose-response studies and biochemical validation. We identified a family of covalent inhibitors that bind a cysteine residue outside the binding site and undergo further chemical modification. Detailed biochemical studies reveal that a specific protein state is inhibited, laying the groundwork for development of more precisely targeted reagents and follow-up functional and preclinical analyses to determine additive or synergistic efficacy as CFTR stabilizers.

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30 March — 16:45–18:30

Symposium 03 - Care for rare: precision medicine to bring innovative therapies to people with rare mutations (a patient organisation symposium)

S3.1 Learning from rarity: lessons from studies on disease-relevant models, In the path from basic defects to personalized therapies

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In Italy, 30% of patients with cystic fibrosis (CF) carry CFTR variants not included among those for which modulators have been approved and are thus defined as "orphan mutations". Some of them have unknown sensitivity to CFTR modulators, while for others their responsiveness has been already demonstrated.

Our study aims at providing each CF patient with the best therapeutic option available by assessing its responsiveness to the different treatments, thus defining its "theratype". The project, relying on the use of patients' nasal epithelial cells is focusing on: 1. understanding the molecular mechanism by which orphan mutations cause CFTR loss of function; 2. defining their responsiveness to CFTR modulators.

Epithelial cells, obtained by nasal brushing, are cultured using a well-established protocol. Approved drugs or compounds under development are tested on nasal epithelia by means of electrophysiological techniques. Molecular analysis of CFTR transcripts is performed by standardized molecular biology procedures; functional and biochemical characterizations are also realized both in native and heterologous systems.

In the frame of our project, we created a network of collaborating CF centres. To date, we have recruited 295 donors. Evaluation of drug responsiveness has been already completed for approx. 200 individuals We have also characterized patients carrying complex alleles: in some cases, the presence of additional variants was unknown. The molecular characterization of CFTR transcripts we performed was crucial to identify the presence of such additional variants impacting the response to CFTR modulators.

So far, our study has demonstrated that a significant fraction of Italian patients carries mutations that can be rescued by modulators, and thus they might benefit from treatment with these drugs. Effective modulators have been identified for more than 50% of the patients. CFTR rescue varies from 5% to 80% compared to that measured in non-CF nasal cells. The study also highlights the existence of poorly responsive mutations that will require novel therapeutic approaches based on optimized modulators or, in other cases, molecular-based strategies.

Interestingly, our study also confirms the importance of the cell background in the evaluation of CFTR modulator effects, as in the case of the mutant G1244E protein, whose processing and function as well as its pharmacological sensitivity, are markedly dependent on the cell context. Finally, our results draw attention to the need for the development of novel potentiators having different mechanisms with respect to ivacaftor to improve channel activity for mutants with severe gating defect.

This work was supported by the Italian Cystic Fibrosis Foundation grant (FFC #10/2021), by the Cystic Fibrosis Foundation grant (PEDEMO20G0), by the Italian Ministry of Health (GR-2018-12367126). The authors thank the people with CF for their participation in this study and their continuous support of their work.

S3.2 *Ex vivo* models / translational models

I Sermet-Gaudelus, E Dreano, A Hatton, M Kelly, I Pranke

INSERM U1151, Institut Necker Enfants Malades, Univeristé de Paris, Center for Rare Disesaes, Cystic Fibrosis and CFTR-Related Diseases, Assistance Publique Hôpitaux de Paris, ERN LUng

Ex vivo models, derived from respiratory or intestinal epithelial cells hold promise for guiding precision medicine and expanding CFTR modulator treatment in patients carrying rare mutations. They require the skills of a central laboratory and provide results around 6 weeks after sampling. The sample can be performed locally, either by rectal biopsy or nasal brushing, and only requires short training of staff. Samples can be shipped to a central laboratory, for expansion, differentiation and biobanking. Importantly, unlike the Fisher Rat Thyroid model which express a single variant, they provide a reproducible response, in a human model carrying 2 different variants, with the possibility to compare different drugs.

Previous studies have shown that direct or indirect quantification of the CFTR-mediated Chloride secretion in patient-derived 2D or 3D epithelial culture is a valuable, easy to implement, surrogate biomarker and that the CFTR functional spectrum in this preclinical model is related to a gradient of CFTR expression at the apical membrane. But we still lack reliable reports showing a good correlation between the level of correction and the respiratory response. Nevertheless, pilot studies suggest that a CFTR activity correction of at least 5% to 10% of the Wild Type is a strong argument to predict a significant respiratory improvement. Such models enable enlarging the list of approved variants for the Elexacaftor/Tezacaftor/Ivacaftor (ETI) combination.

Such data are particularly relevant in the context of emergent therapies that rescue CFTR to identify subjects with CF who may benefit from CFTR-modulating drugs. This also provides insight into the molecular mechanism of specific variants, i.e; for splicing variants, study of the transcript level and length in correlation with CFTR activity correction, or search for complex alleles modulating the response of missense variants. Finally, and most importantly, they may also be very useful to test different drug combination for a specific genotype, on the patient specific background. This new theranostics classification, based on functional "theratype", driven by patient-specific responses, provides a rationale for a personalized medicine strategy tailored for every patient with CF.

S3.3 Regulatory aspects, hurdles and needs on the way to realize personalized medicine in CF

Marco Cipolli

Cystic Fibrosis Center, Verona, Italy

To date, the therapies able to correct the CFTR genetic defects cover 70-90% of CF patients, depending on the frequency of F508del mutation in their countries of origin. In some countries, such as Italy and Israel, the frequency of CF patients carrying rare mutations other than F508del is higher compared, for example, with countries from northern Europe or US.

In this historical period there are many research centers, start-ups and companies engaged in the development of molecules able to correct the CFTR defect due to rare mutations.

However, in this scenario, the transition from the preclinical model to human trials is particularly complex. Unfortunately, clinical trials (and personalized medicine) are challenging due to the rarity of these CFTR mutations, the low prevalence, and the ever-improving treatment outcomes.

There are several limitations to the applicability of traditional population-based Clinical Trials in CF patients with rare CFTR mutations. The most important limitation is related to sample size. In conventional phase II--III trials, hundreds or thousands of patients are usually enrolled. The number of patients that should be enrolled in a particular trial is assessed through the process of power analysis based on frequentist theory. The number of patients required to prove the expected clinical effect statistically significant is calculated.

Regarding hurdles, we have to consider for exaple the patients' geographical dispersion that requires multicentre and multinational collaboration, introducing additional regulatory and funding obstacles. Travel to research centres may be impossible. Some solutions propose monitoring patients remotely, setting up community centres to include patients in trials who would otherwise be unable to access them. Effective recruitment is often supported through partnership with patient organisations, when available, and through the use of patient registries and centres of expertise.

However, the perspectives on the whole issue of rare diseases and personalized medicine have been changing in the last years. Furthemore different experimental models for CF, passing from the idea of population studies to individual studies need to be proposed and tested.

The development of clinical trials for CF patients with rare mutations therefore requires a concerted approach by all stakeholders. New approaches in the arrangement of clinical studies will be discussed, with particular emphasis on those designed for ultra-rare mutations.

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S3.4 Pan-ethnic characterization of CFTR variants

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Introduction: In cystic fibrosis (CF), small molecule therapies targeting the basic CFTR defects have shown clinical benefits, but therapeutic responses are largely dependent on the patients' *CFTR* genotype. Although CF is most prevalent in people with European ancestry, the distribution of dominant *CFTR* or CF-causing variants vary across ancestries. Thus, characterizing the broad spectrum of pathogenic and non-pathogenic *CFTR* variants across ancestries is critical for revolutionizing molecular diagnoses of CF.

Methods: We analyzed 454,857 whole-exome sequences generated by UK Biobank (UKBB) to characterize the diversity of *CFTR* variants across ancestries. We used the ancestry assignment done by the pan-UKBB to investigate the frequency of *CFTR* variants in Africans, American, Central South Asian, East Asian, European, and Middle Eastern populations. We then performed an overlap analysis to decipher ancestry specific *CFTR* variants. CF-causing variants were then annotated using the CFTR2 database before populating the frequency distribution across ancestries.

Results: Overall, we detected over 4,000 *CFTR* variants from all whole exome sequences analyzed in this study. Many of these variants have never been reported in CF. The highest number of *CFTR* variants were detected in Europeans [n=3,192] while American group had the least number of *CFTR* variants [n=1507]. We found several variants specific to each ancestry, with Europeans having most of the unique variants [n=2212] while the American group has the least number of unique *CFTR* variants [n=23]. F508del was the most prevalent CF-causing variant found in all ancestries, except in East Asia, where V520F was the most prevalent. Participants undiagnosed as CF but harboring two CF-causing variants (n>10) reported phenotypes significantly (adjusted $p<0.05$) associated with CF pulmonary phenotypes.

Conclusion: Most CF-causing variants have been characterized by investigating European populations. The identification of several unique uncharacterized variants in other ancestries warrants the need for further studies to delineate their functional relevance. The presentation of classical CF phenotypes seen in some non-CF diagnosed participants, with more than one CF-causing variants, indicates they may benefit from current CFTR therapies.

Disclosure: All authors are employees of AbbVie. The design, study conduct, and financial support for this research were provided by AbbVie. AbbVie participated in the interpretation of data, review, and approval of the publication.

31 March — 08:45–10:30

Symposium 4 – Mucus and mucins

S4.1 Mucin in High Resolution

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Insights into molecular structure and mechanism have driven major advances in biology and medicine over the past few decades. Mucosal biology and the treatment of mucus-associated diseases may similarly benefit from an understanding of the glycoprotein structures that constitute mucus. However, the enormous sizes and configurational and compositional heterogeneity of key mucus molecules, such as the gel-forming mucins, have complicated structural analysis. Enabled by recent technical advances in cryo-electron microscopy and the observation that recombinant segments of gel-forming mucins form orderly structures representing intermediates in the assembly of disulfide-bonded polymers under conditions that mimic the Golgi apparatus or secretory granules, we have made headway in determining high-resolution structures of intestinal (MUC2) and respiratory (MUC5B) mucins. Mucin fragment structures have begun to reveal the mechanism for mucin polymerization, the role of O-glycosylated threonine- and serine-rich regions, and a special function for the small, disulfide-rich CysD domains that are embedded in multiple copies in the major gel-forming mucins. Our goal is to use structural and mechanistic information obtained from the study of recombinant mucin fragments to shed light on the complex behavior of intact mucins *in vivo* in health and disease states. Specifically, we aim to determine how the Ångstrom- and nanometer-scale data obtained in our experiments relate to the micron- and millimeter-scale appearance and biophysical properties of mucins relevant to their function at mucosal surfaces.

S4.2 Progressive development of abnormal mucus precedes chronic *P. aeruginosa* infection in the cystic fibrosis rat

Susan Birket

University of Alabama at Birmingham, Birmingham, United States

Cystic fibrosis lung disease is characterized by increased buildup of viscous mucus, obstructing small airways, enabling bacterial infections establish early in life, and leading to lung function decline. Mucus in the airways is a part of the normal innate immune defense that becomes dysregulated in the CF lung. Major components of mucus buildup include the secreted airway mucins MUC5B and MUC5AC, extracellular DNA, and bacterial and host cell debris. Understanding the mechanisms that lead to excess and hyperviscous mucus accumulation in the airways is crucial to development of supportive therapies, as well as to assess the overall efficacy of CFTR-directed therapies.

Rat models of CF have been developed to study these mechanisms. The CFTR-KO rat develops mucus accumulation and secretion progressively, with relatively normal lungs at 1 month of age, including appropriate mucociliary clearance and similar amounts of secreted mucins in the airway compared to wild-type (WT) age-matched controls. As the CF rats age to 6 months, they develop goblet hyperplasia, increased area of airway submucosal glands, and increased concentrations of Muc5b and IL-1β in the airway, accompanied by decreased mucociliary transport and increased mucus effective viscosity. Additionally, mucus becomes adherent to the airway epithelial surface. As the rats reach 6 months of age, and develop abnormal mucus parameters, they also become susceptible to chronic infection with *P. aeruginosa*. Rats infected with a mucoid clinical isolate of *P. aeruginosa* at 2 months of age, when mucus transport and Muc5b concentrations are no different than WT, are able to clear infection within 7 days following administration. Bacterial eradication is accompanied by a complete resolution of inflammatory and mucus responses to infection. However, when 6 month old CF rat are infected with the same strain of *P. aeruginosa*, they remain infected, with detectable CFUs in the 10^6 range until 56 days post-infection, becoming chronically infected. Chronic infection in the CF rats is accompanied by small airway occlusions and an increase in Muc5ac in addition to Muc5b.

Because Muc5b is abnormal prior to exposure to *P. aeruginosa*, we hypothesized that normalization of Muc5b concentrations in the airway would ameliorate the development of chronic infection. CF rats were treated with Muc5b-siRNA, compared to scramble control, prior to exposure to bacteria. Muc5b-siRNA dosed at 40 μg normalized Muc5b concentrations to that of the WT littermate controls, while scramble did not alter Muc5b concentrations in the CF rat lung. Pre-treatment with Muc5b-siRNA followed by infection with *P. aeruginosa* did not alter acute (day 3) infection outcomes, but did result in significantly lower CFUs, fewer neutrophils, and fewer small airway impactions at 14 days following infection. This suggests that the development of chronic infection may be impaired or delayed when Muc5b production is impaired.

S4.3 The distinct components of airway mucus

Brendan Dolan[1], Lauren N. Meiss[1], Ana M. Rodriguez-Piñeiro[1], Florian Jaudas[2], Nikolai Klymiuk[2], Andrea Bähr[2], Gunnar C. Hansson[1], Anna Ermund[1]

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Airways and lungs are exposed to airborne pollutants, microbes and particles with every breath. In normal lungs, inhaled pollutants, microbes and particles are captured by mucus and moved cephalad by the coordinated movement of cilia. However, in cystic fibrosis, the movement of mucus is impaired, resulting in accumulation of microbes and induction of an inflammatory response. The normal mucus is transparent due to high water content and studying the mucus in its native form is challenging. However, in order to understand normal mucus as well as how it is affected in cystic fibrosis, mucus must be studied in its native environment, in live tissue or it will lose its inherent structure and interactions. Using antibody staining and fluorescent lectins, we have demonstrated in excised tissue from newborn piglets that mucus bundled strands consisting of MUC5B from submucosal glands are important for airway clearance and are stagnant in cystic fibrosis piglets. Furthermore, we have identified mucus threads secreted from airway epithelial goblet cells as responsible for collection of inhaled particles. The treads moved faster than the bundles and their transport was stimulated by acetylcholine, whereas the bundles were stopped by cholinergic stimulation, suggesting differential function in normal airways. The bundled strands from submucosal glands become coated by mucus from surface goblet cells and are retained on the surface by the goblet cells in cystic fibrosis. This stagnation of bundled strands in newborn cystic fibrosis piglets leads to decreased clearance of particles, microbes and mucus, resulting in the increased bacterial colonization and hypoxia observed in cystic fibrosis, as well as the increased inflammatory tone with neutrophil infiltration and lack of resolution. This in turn leads to a stratified mucus layer in adult cystic fibrosis patients, attempting to keep the microbes at a distance from the epithelium. Thus, normal mucus is easily movable and contains a defined set of proteins analyzed by mass spectrometry-based proteomics. In cystic fibrosis before overt inflammation can be observed, bundled strands are immobile whereas threads are unaffected. In terminal cystic fibrosis lung disease, mucus forms a striated layer to keep the bacteria away from the epithelium, as in the normal colon.

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S4.4 Low molecular weight alginate oligosaccharides enhance the diffusion of lipid-nanoparticles through cystic fibrosis mucus

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The airway mucus barrier is a major barrier for lipid nanoparticle (LPN) based therapies targeting the human lung epithelium. Mucociliary clearance removes foreign agents from the lung while chronic airway inflammation, often observed in cystic fibrosis (CF), increases the thickness and the viscosity of mucus barrier, which leads very low drug delivery efficiency.

We are developing novel receptor-targeted nanocomplexes (RTNs) for delivery of *in vitro* transcribed mRNA (IVT mRNA) or siRNA to lung epithelial cells as a therapy for cystic fibrosis. The RTN formulation comprises a mixture of cationic lipids and epithelial receptor-targeting peptides yielding cationic nanoparticles. While effective transfection agents, cationic RTNs have poor mucus diffusion properties which could limit their in vivo transfectiojn properties. To increase their mucus penetration efficiency, we combined RTNs with negatively charged low molecular weight alginate oligosaccharides OligoG and OligoM (Algipharma), (Mn 3200 g/mol), anionic and linear structures composed of (1-4) linked β-D-mannuronic acid (M) and its C-5 epimer α-L-guluronic acid (G). Their effects on RTNs were assessed in a mucus diffusion assay using a mucus barrier model comprising CF mucus on polyester membrane transwell inserts with 3.0 μm pores and 50 mM Tris buffer pH 8.0 was filled in the basolateral side in 24-transwell plates. OligoG and OligoM were added directly to the mucus layer before RTNs were added or were formulated with the RTNs.

We first investigated the effects of charge on the mucus penetration efficiency of RTNs containing IVT mRNA, non-PEGylated cationic RTNs (DOTMA/DOPE) and ICAM-1 receptor targeting peptide E displayed very low mucus diffusion rate. The effects of OligoG and OligoM on RTN diffusion were investigated by adding the reagents to mucus, or by formulating with the RTNs themselves. The RTNs prepared by incorporation of OligoG or OligoM into the cationic RTN were found to have anionic surface charge (zeta potential) while the RTNs containing siRNA with OligoM were smaller than those without. OligoG added to cationic mRNA RTNs dramatically improved their diffusion in mucus due to their anionic charge and smaller size. The alginates added to mucus before addition of cationic RTNs also improved the diffusion rate but not as much as those formulated in RTNs. RTNs containing siRNA instead of mRNA, both approaches of adding OligoG and OligoM to mucus or incorporating into cationic RTNs improved the diffusion rate. The alginates added to mucus improved the diffusion rate of the RTNs whilst the reagents formulated in the RTNs did not. However, the improvement was not as high as those in the mRNA RTNs.

In conclusion, OligoG and OligoM promote mucus penetration of cationic RTNs containing siRNA and mRNA, with particlualr benfit for those containing mRNA. These alginates appear to reduce the interaction of RTNs with mucins, and increase fluidity of the mucus itself. Especially OligoG formulated in cationic mRNA RTN significantly increase the penetration efficiency and may enhance the transfection efficiency in CF lungs.

P21

S4.5 Structure of domains in polymerizing mucins and their importance for the formation of normal and CF mucus

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The major constituent in all mucus is one or several gel-forming mucins. In the intestinal tract, it is only the MUC2 mucin whereas the respiratory tract normally have two mucins, the MUC5B and the MUC5AC. All these mucins are built by central PTS sequences that after dense O-glycosylation become extended, stiff mucin domain rods interrupted by CysD domains. The CysD domains are around 100 amino acid with five disulfide-bonds that stabilize a stalk made up by six antiparallel β-strands. The top of this stalk carries two flexible loops that extend outside the surface formed by the glycans taking part in pH-dependent dimerization.

The N-terminal part of these mucins are made up 3.5 von Willebrand D (VWD) assemblies, each built by a VWD domain, a C8, and a TIL domain. The first two WVD assemblies are important during intracellular packing in the goblet cell mucin granule, but it is the dimerized third VWD3 assembly that is important for the assembled mucus. The MUC5B mucin is only forming the disulfide-bonded dimers and thus linear polymers. In the MUC5AC on the other hand, the VWD3 forms additional non-covalent interactions to generate net-like polymers, interactions that vary in strength depending on genetic SNP variability.

The C-terminal part of these mucins are made up by one VWD assembly, 3.5 VWC domains and finally a C-terminal cystine knot domain. The structure of the MUC2 C-terminus has been determined and show as the other mucin a tightly held together extended dimeric structure. All are dimers due to the cystine-knot domain, but the MUC2 is also stabilized by an extra disulfide bond before the C-terminal VWD.

Interactions between these different domains determines the packing of the mucins at low pH intracellular and the formation of the mature mucus after secretion and expansion as triggered by bicarbonate and increased pH.

P23

S4.6 Sputum viscoelasticity is driven by concentration in organic components

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Sputum from Cystic Fibrosis (CF) and Non-Cystic Fibrosis Bronchiectasis (NCFB) patients have high viscoelastic properties which complicates its clearance leading to airways obstruction and infection. Sputum viscoelasticity has been shown to correlate to solid fraction. However, it remains unclear whether this correlation is primarily driven by organic (mucins, DNA, cell debris, etc.) or inorganic (salts) content. Here we quantify separately these two influences in CF and NCFB sputum samples.

CF and NCFB adult sputum samples were collected from the “Centre de Ressource et de Compétence de Mucoviscidose" (CRCM) in the Grenoble Alpes University Hospital (CHU). The study was approved by the Comité de Protection des Personnes (case number 20.09.08.61213). Expectorations were induced by drainage with the help of a physiotherapist and freeze dried prior to characterisation. Sample’s solid concentration was measured by drying at 37°C during 24h and weighting it in a precision balance, and salt concentration was obtained by conductimetry. Furthermore, rheometry was measured for each sample at low and high deformation obtaining the viscoelastic modulus (G*) and the critical strain and stress (and), respectively. Finally, an NCFB expectoration was gradually diluted at isosalinity to assess if the variability between patients could be retrieved by simply tuning the sample’s hydration.

Total solid concentrations of CF (n=13) and NCFB (n=15) samples ranged between 8.42 and 104.29 mg/mL and 21.3 and 86.96 mg/mL respectively, and the salt amount was 5.62 ± 0.44 mg/mL in CF and 7.94 ± 1.73 mg/mL in NCFB expectorations.

We observed a linear correlation between G* and the organic solid concentration, and no significant dependence to salt concentration.

Remarkably, the correlation between G* and organic solid concentration was identical between CF and NCFB. Moreover, the hydrated sample also fell onto the same correlation, which suggests that the inter-sample’s variability in G* is merely caused by the proportion of water and organic solids, regardless of the detailed composition of the sample.

This study has therefore confirmed the influence of organic solid concentration on sputum’s viscoelasticity while showing no evidence of salt influence.

31 March — 11:00–12:45

Symposium 5 – Inflammation & Modulators

S5.1 The CF Airway Inflammatory Milieu Enhances the Efficacy of CFTR Modulators

Carla M. P. Ribeiro, Kenichi Okuda, Charles R. Esther Jr., Martina Gentzsch

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Defective CFTR biogenesis and function in airways of people with cystic fibrosis (pwCF) causes airway dehydration and impairs mucociliary clearance, leading to chronic airway infection and inflammation. Most pwCF have at least one copy of the F508del CFTR mutation, which is translated into a mutated protein retained in the endoplasmic reticulum and degraded by the proteosomal pathway. The availability of CFTR modulators, e.g., correctors (lumacaftor, tezacaftor, elexacaftor) that promote the transfer of F508del and other mutated CFTR (i.e., N1303K) to the apical membrane, and potentiators (ivacaftor) that increase CFTR activity, has dramatically altered CF care. Triple therapy with two correctors and one potentiator yields substantial clinical benefits, including reduction of some airway inflammatory markers. However, recent evidence suggests that the CF airway inflammatory milieu may impact the efficacy of CFTR modulators. This presentation will review recent studies utilizing translational models of CF human bronchial epithelial (HBE) inflammation to evaluate the impact of the CF airway environment on modulator-induced mutant CFTR (e.g., F508del and N1303K) rescue.

Inflammation was induced by exposing primary CF HBE cultures to relevant CF inflammatory stimuli, e.g., supernatant from mucopurulent material (SMM; harvested from excised CF lungs) or bronchoalveolar lavage fluid (BALF; from pediatric pwCF). Inflammation enhanced the biochemical and functional F508del CFTR rescue by double or triple CFTR modulator therapy, and increased the activity of N1303K CFTR and its rescue by triple modulator therapy. Notably, interleukin-1β (IL-1β), a predominant cytokine in SMM, also increased the CFTR rescuing efficacy of modulator therapy, and the IL-1 receptor antagonist Anakinra blocked the IL-1β-dependent responses. These data indicate that SMM-enhanced CFTR rescue is mediated, at least in part, by IL-1β-dependent signaling.

The enhancing effect of inflammation on CFTR rescue will also be discussed regarding our recently obtained correlation between airway inflammation and ivacaftor-induced improvements in chest CT scores, and data from other colleagues indicating a beneficial effect of inflammation on clinical outcomes in pwCF.

S5.2 Molecular Targets for Anti-Inflammatory Therapies

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PwCF treated with Highly Effective Modulator (HEM) Therapy show significant improvements in lung function and reduced frequency of infective exacerbations. Contradictory results have been reported on the effects of inflammation on mCFTR rescue and on the recovery of a physiological fluidity of CF airway surface liquid. Interestingly, clinical trials of PwCF treated with HEMs showed a variable decrease of concentration of some soluble inflammatory markers in blood or sputum. Whether this partial decrease represents a solid proof that HEMs are sufficient to halt the progression of lung tissue damage produced by a neutrophil-sustained excessive inflammation is under scrutiny, considering that lung function slowly worsens also in HEMT-treated PwCF. Thus, combining HEMs with newly designed anti-inflammatory (AI) drugs able to counteract lung tissue damage produced by chronic inflammation would represent an important therapeutic strategy, also in the era of HEMT.

The main approaches to tackle CF lung inflammation include: (a) the neutralization of neutrophil products, such as elastase and reactive oxygen species; (b) the control of the excessive neutrophil recruitment in the airways mucosa; (c) the augment of counter-regulatory pathways, such as resolvins. The pro-inflammatory role of different molecular targets activated by host-pathogen interactions in CF bronchial epithelial cells, including MyD88-dependent pathway or intracellular calcium transients dependent on its release from intracellular stores, via activation of phospholipase C isoforms, or influx through plasma membrane refill systems, e.g. Transient Receptor Potential Ankyrin 1 Channels, will be briefly introduced.

In terms of pre-clinical pharmaceutical developments, the design and synthesis of chemical analogues of the prototype furocoumarin 4,6,4'-trimethylangelicin (TMA) has been completed in order to obtain a safe AI molecule, deprived of photoreactivity, mutagenicity and cytotoxicity *in vitro*. Molecules passing the safety tests *in vitro* have been subjected to pharmacokinetics studies in mice, with different formulations for oral administration to improve their bioavailability. Molecules with promising *in vivo* bioavailability were then screened for their ability to inhibit the interaction of the transcription factor NF-kappaB with its consensus sequence in the promoter of the neutrophil chemokine IL-8/CXCL8. TMA analogues with the highest inhibitory potency of NFkappaB/IL-8 binding were further tested in a model of CF bronchial epithelial cells challenged *in vitro* with TNF-alpha or with *P.aeruginosa*, to select the most active ones in reducing the secretion of IL-8/CXCL8 and of pro-inflammatory cytokines. Next, the AI activity of the most promising TMA analogue GY971 has been confirmed in a model of *P.aeruginosa*-infected murine lung model *in vivo*, where reduction of inflammation has been obtained without suppression of the immune defenses and untoward increase of bacterial load.

In conclusion, the limitations of the presently available broad-based AI drugs, such as corticosteroids and ibuprofen, will be overcome by newly designed molecules that tackle CF specific pathways of inflammation, with the mandatory goal of intervening to limit inflammatory tissue damage without blunting the immune defenses against infection.

S5.3 Inflammation, resolution and repair in the era of highly effective modulator therapy for CF

Robert D Gray[1,2]

[1]University of Edinburgh Centre for Inflammation Research. , [2]Scottish Adult CF Service, Western General Hospital Edinburgh

Highly effective CFTR modulators (HEMT) offer an effective treatment that improves multiple outcomes for the 90% of people with CF eligible for treatment with these drugs. A major feature of CF lung disease is inflammation and consequent lung damage. To date inflammation in CF has been studied widely but not fully understood. Key features of CF inflammation include increased levels of airway and systemic biomarkers of inflammation such as calprotectin and pro-inflammatory cytokines, as well as fundamental changes in immune cells such as prolonged neutrophil lifespan and the presence of pro-inflammatory monocytes and macrophages. We will review previous studies that have investigated the impact of CFTR modulators on inflammation and will present new data investigating the impact of HEMT on systemic inflammation in the early stages of treatment and how this may signal inflammation resolution. Resolution of inflammation is the first step towards tissue repair and further data from patients after 1 year of HEMT therapy suggests on-going inflammation resolution. The impact of prolonged inflammation resolution on potential lung tissue repair is presently unknown but imaging from a cohort of people receiving HEMT show evidence of tissue remodeling. We are now investigating the role of basal cell dysfunction in the CF airway and how interplay of the immune system and basal cells may be key to long lasting tissue repair in CF.

P36

S5.4 Pulmonary ketogenesis promotes tolerance to *P. aeruginosa* infection in the CF lung

Sebastian Riquelme, Kira Tomlinson, Ying-Tsun Chen, Alice Prince

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Introduction: *P. aeruginosa* is a major opportunist that causes chronic respiratory infection in patients with CF. *P. aeruginosa* readily adapts to the CF lung to coexist with its host, establishing a favorable airway microenvironment that tolerates the pathogen burden. However, exactly how *P. aeruginosa* promotes this milieu remains poorly understood. Ketone bodies are typically liver metabolites that promote tissue homeostasis not only by fueling cellular bioenergetics, but also by desensitizing phagocytes to infection. Here, we established that *P. aeruginosa* exploits host ketogenesis to generate an airway milieu that tolerates infection in the CF lung.

Methods: In a murine model of pneumonia, we studied tolerance to a collection of 17 CF host-adapted *P. aeruginosa* isolates and *P. aeruginosa* PAO1 as control. Airway and systemic ketones were quantified by metabolomics. The ketogenic program of each lung cell during *P. aeruginosa* pneumonia was analyzed by single-cell RNA-Seq. Airway accumulation of cytokines and effector cells were examined by ELISA and multi-color flow cytometry, respectively. To establish a ketogenic environment in the lung, we fed animals a fatty acid-rich, carbohydrate low ketogenic diet in comparison to a fatty acid-low, carbohydrate rich control diet.

Results: Both the CF host-adapted *P. aeruginosa* isolates and PAO1 triggered airway accumulation of ketone bodies during pneumonia. The ketones induced by the CF host-adapted *P. aeruginosa* isolates were mainly derived from lipofibroblasts, whereas those associated with acute PAO1 infection were produced by the liver. We confirmed the presence of ketone bodies in airway fluids from CF subjects. We recapitulated this ketone-rich environment by feeding mice a ketogenic diet, and found that the CF host-adapted *P. aeruginosa* isolates exploited this milieu to suppress the inflammatory response to infection. Interestingly, we observed that exposure of PAO1 to ketone bodies limited its ability to expose LPS on the surface, which not only reproduced the less inflammatory phenotype found in the CF isolates, but also contributed to the establishment of tolerance to infection.

Conclusions: Our findings demonstrated that CF host-adapted *P. aeruginosa* isolates co-opted metabolic routes associated with preservation of airway bioenergetic function to both adapt to the lung and trigger tolerance to disease, such as ketone bodies.

Funding: SAR is funded by R35GM146776 and CFF RIQUEL21I0. AP is funded by 5R35HL135800 and 003028G221.

P16

S5.5 Differential neutrophilic responses to *Pseudomonas aeruginosa* reveal novel protein-based treatments relevant to cystic fibrosis

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University of Guelph, Molecular and Cellular Biology, Guelph, CA

Cystic Fibrosis (CF) has devastating effects on approximately 70,000 patients worldwide by impeding the transport of fluids from the lungs, leading to chronic inflammation and persistent microbial infections. As a first line of defense against such infections, the host will produce and recruit immune cells (e.g., neutrophils); however, in CF patients, mutations within the cystic fibrosis transmembrane conductance receptor (CFTR) (e.g., ΔF508) render immune cells ineffective at clearing such infections, leading to life-threatening disease. Specifically, CF-associated neutrophils demonstrate impaired effector function compared to wild type neutrophils, causing altered antimicrobial protein and enzyme production. Such altered neutrophil activity in the lungs can permit the adaptation and survival of bacterial pathogens, including *Pseudomonas aeruginosa,* as well as the formation of biofilms, which are resistant to antimicrobial treatments. Presently, our understanding of the interactions between neutrophils and *P. aeruginosa* in a CF model are limited, reducing our ability to effectively treat the host and clear the infection.

In this study, we apply state-of-the-art mass spectrometry-based proteomics to define a baseline of core proteome signatures and unique protein profiles between wild-type (HL60-WT-derived) and CF-associated (HL60-ΔF508-derived) neutrophils under uninfected conditions. We identified 105 host proteins with a significant increase in abundance in the wild-type neutrophils compared to ΔF508 neutrophils, including neutrophil defensin 3 and gamma-enolase. Conversely, we observed 65 proteins with a significantly higher abundance in ΔF508 neutrophils compared to WT, such as galectin 1 and argininosuccinate synthase. Next, to evaluate how neutrophil protein production impacts clearance of *P. aeruginosa* biofilms, we performed quantitative proteomics profiling on WT and ΔF508 neutrophils following incubation with *P. aeruginosa* biofilms. Here, our experiments identified many neutrophil proteins with significantly altered abundance profiles across the conditions, including bactericidal permeability-increasing protein and cathepsin G, which were elevated in WT neutrophils during infection. Importantly, our dual perspective proteomics profiling (i.e., detecting both host and pathogen proteins in a single experiment) also revealed significant changes in the bacterial response profiles between the tested neutrophils, suggesting that the interactions between neutrophils and *P. aeruginosa* biofilms are also critical to driving infection. We support our observations with assessment of the WT vs. ΔF508 neutrophils in clearing *P. aeruginosa* biofilms using crystal violet assays and colony forming unit (CFU) counts to quantify biofilm disruption. Next, host proteins with known or predicted roles in bacterial clearance (e.g., enzymes, immune-associated) will be prioritized for *in vitro* purification and further characterization to identify candidates' leading to enhanced biofilm disruption. To further analyze protein candidates' influence on host-pathogen interactions scanning electron microscopy (SEM) and biofilm disruption assays will be performed to validate these findings.

Together, this work enhances our understanding of biological mechanisms underpinning the host's ability to clear bacterial infections. Further, these findings support the discovery of potential novel therapeutic strategies to clear *P. aeruginosa* biofilms and aim to improve the quality of life for patients and their families living with CF.

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S5.6 Fertility deficiency in female cystic fibrosis patients: determining the role of the uterus' endometrium using cutting-edge organoid models

Ellen De Pauw[1], Marjolein M Ensinck[2], Stefan Timmerman[3], Marianne S Carlon[2,4], Hugo Vankelecom[1]

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Introduction/objectives: Female CF patients suffer from sub- to infertility, often facing problems to become pregnant. Underlying reasons remain understudied. In particular, it is largely unknown whether dysfunction of the endometrium is involved, the womb's inner lining and key tissue for embryo implantation and development. Although pregnancy rates have increased since the new-era CFTR modulator therapies, their direct impact on the female reproductive tract, and particularly the endometrium, is unknown. These gaps in the CF domain are mainly due to lack of appropriate study models. Therefore, we develop endometrium-derived, tissue-mimicking organoid models which are applied to decode the role of the endometrium in CF fertility deficiency and the impact of clinical CFTR modulators on endometrial (dys-)function.

Methods: We establish endometrial organoids (EMO) from both *Cftr*^{tm1Eur} mouse and human CF patients. The organoids are molecularly and functionally compared to healthy EMO to uncover aberrations that underlie CF fertility deficiency. FIS, Ussing chamber and HS-YFP quenching assays are used to measure CFTR function in the organoids and their response to CFTR modulators. To assess differences in endometrial responsiveness to estrogen (E2) and progesterone (P4), which *in vivo* regulate the estrous or menstrual cycle, EMO cultures are exposed to defined E2/P4 regimens. Expression of CFTR, endometrial functionality/fertility markers as well as inflammatory genes is investigated by RT-qPCR and immunofluorescence staining. Multiple CF tissues are indeed characterized by a (hyper-)inflammatory phenotype which in the endometrium would be an important contributor to dysfunction.

Results: First, we established EMO from *Cftr*^{tm1Eur} mice, which show a smaller lumen than wildtype (WT) EMO and do not swell in the FIS assay, both validating the CFTR defect. When treated with CFTR modulators, organoid swelling is rescued. Currently, we are comparing expression of endometrial functionality/fertility markers and inflammatory genes in CF *versus* WT EMO, as well as their responsiveness to E2 and P4 (mimicking the different estrous cycle phases).

Second, we develop(-ed) organoids from CF patient endometrium. Before, we have shown that EMO from healthy endometrium can reliably reproduce all menstrual cycle phases under defined E2/P4 exposure. These organoids show phase-dependent *CFTR* expression similar to *in vivo*. Moreover, functionality of the expressed CFTR (in healthy EMO) was confirmed by HS-YFP quenching and Ussing chamber assays. Currently, we are deciphering whether and how menstrual cycle phases, including the embryo receptivity stage, are different between CF and healthy EMO. First experiments show dissimilarities in the proliferative and secretory phase, such as increased apoptosis and decreased fertility marker expression, respectively, thus providing a first indication of aberrant hormonal response in human CF endometrium. Now, the impact of CFTR modulators is being assessed.

Discussion: We establish(-ed) organoid models from CF endometrium as novel and powerful tools to gain insight into the endometrial factor in CF fertility deficiency. Importantly, the organoids are highly apt to explore the impact of CFTR modulators on the endometrium, at present unknown. Moreover, our study has the potential to reveal paths toward restoring reproductive fitness in CF patients, which can be tested using the organoids as (drug) screening platform.

01 April — 08:45–10:30

Symposium 6 – ABC proteins and their response to modulators

S6.1 Development of Targeted Pharmacotherapy to Correct ABCB4 Deficiency

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In the liver, a series of ATP-Binding Cassette (ABC) transporters (ABCB4/MDR3, ABCG5-ABCG8 and ABCB11/BSEP) catalyze the generation of bile and their dysfunctions lead to rare, evolutionary diseases for which there is often no therapy. Our research is focused on *ABCB4* gene encoding the biliary phospholipids transporter ABCB4/MDR3, which deficiency causes a wide range of biliary diseases including Progressive Familial Intrahepatic Cholestasis type 3 (PFIC3). PFIC3 is a severe disease that typically starts in childhood and can be lethal in the absence of liver transplantation. In this context, the development of alternative therapies is a major challenge. Impaired biliary phosphatidylcholine excretion by hepatocytes results in the formation of cytotoxic bile, leading to cellular damage, inflammation and cholestasis. So far, 500 *ABCB4* gene variations have been identified in patients with biliary diseases including missense, nonsense and splice mutations. Our research projects are based on a tight interplay between theoretical and experimental approaches to investigate how sequence variants in the *ABCB4* gene provide insight into how mutations impact the function of the ABCB4 protein. We are also developing specific therapeutic approaches, including drug repositioning to correct ABCB4 deficiency. We have unraveled several mechanisms by which *ABCB4* missense variations cause diseases and introduced the first functional classification of these variations based on their impact on the production, traffic, activity or stability of the protein. This presentation will focus on personalized therapeutic strategies that we developed to correct the pathological consequences of several ABCB4 variations identified in PFIC3 patients.

S6.2 Understanding conformational transitions favoring CFTR NBD1 unfolding to design conformation-specific therapeutics

Daniel Scholl[1], Maud Sigoillot[1], Marie Overtus[1], Rafael Colomer Martinez[1], Chloé Martens[1], Yiting Wang[2], Els Pardon[3,4], Toon Laeremans[3,4], Abel Garcia-Pino[5], Jan Steyaert[3,4], David N. Sheppard [2], Jelle Hendrix[6,7], Cédric Govaerts *[1]

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Cystic Fibrosis (CF) is a common lethal genetic disorder caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) anion channel. The predominant CF-causing mutation, F508del, is located in the first nucleotide-binding domain (NBD1) and leads to misfolding and degradation of CFTR through molecular events that remain elusive. Here, we demonstrate that NBD1 of CFTR can adopt an alternative conformation that departs from the canonical NBD fold previously observed for CFTR and other ATP-binding cassette (ABC) transporter proteins. Crystallography studies reveal that this conformation involves a topological reorganization of the β-subdomain of NBD1. We observe that this alternative state is adopted by wild-type CFTR in cells, where it leads to enhanced channel activity. Single-molecule fluorescence resonance energy transfer microscopy shows that the equilibrium between the conformations is regulated by ATP binding. Under destabilizing conditions, however, this conformational flexibility enables unfolding of the β-subdomain. Our data indicate that in wild-type CFTR switching to this topologically-swapped conformation of NBD1 regulates channel function, but, in the presence of the F508del mutation, it allows domain misfolding and subsequent protein degradation. Our work provides a framework to design conformation-specific therapeutics to prevent noxious transitions.

S6.3 Design of novel peptides potentiators based on protein-protein interactions

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Protein-protein interactions regulate multiple aspects of CFTR biogenesis, such as its folding, activity and degradation. Numerous CFTR partners have been identified and their effect on CFTR characterized. Targeting the amount or activity of the partner can affect CFTR. Alternatively, the effect of the interaction, in some cases, can be reproduced by peptides mimicking the interface of CFTR interacting protein.

As has been shown previously, the CBb subunit of crotoxin from *Crotalus durissus terrificus* which is a β-neurotoxin with phospholipase A2 activity (PLA_2), interacts with the human ΔF508CFTR chloride channel (Faure et al, JMB, 2016). By direct binding to the nucleotide binding domain 1 (NBD1) of ΔF508CFTR, CBb acts as both a potentiator increasing chloride channel current and a corrector of the ΔF508CFTR trafficking defect inside the cells. Nonetheless, the complex structure and toxicity of CBb prevents its potential therapeutic applications.

In the current studies, we applied a structure-based in silico approach to design a series of peptides mimicking the CBb-ΔF508NBD1 interface (Ravatin et al, JMB, 2023). These were based either on the CBb sequence in contact with ΔF508NBD1 or designed to reproduce the 3D spatial orientation of key aromatic amino acids involved in the contact interface. The designed peptides bind to the same region as CBb on ΔF508NBD1 as assessed using surface plasmon resonance and potentiate chloride channel activity measured by both automated and manual whole cell patch-clamp measurements. Certain peptides also show an additive effect towards the clinically approved VX-770 potentiator. Mutation of CFTR amino acid Y625 prevented peptide induced potentiation, while VX-770 potentiation was not affected, confirming the peptide region of interaction.

The identified CF therapeutics peptides represent a novel class of CFTR potentiators and illustrate a strategy leading to reproducing the effect of specific protein-protein interactions.

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S6.4 A novel CFTR corrector displaying a unique mode of action

Azib Hagos[1], Corina Balut[2], Aya Saleh[2], Ashvani Singh[2], Ineke Braakman[1], Peter van der Sluijs[1]

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Programme note: the details are not authorised for inclusion

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S6.5 Use of computer simulations to investigate ivacaftor-induced conformational changes in CFTR

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Treatment of cystic fibrosis has been transformed by orally administered drugs that directly target the cystic fibrosis transmembrane conductance regulator (CFTR). Ivacaftor, the first clinically approved CFTR potentiator, binds CFTR in a cleft formed by three transmembrane segments at the lipid-protein interface, which includes the unstructured region of transmembrane segment 8[1,2]. However, the conformational changes initiated by ivacaftor binding to CFTR remain largely unknown. Here, we investigate how the ivacaftor-binding site is allosterically coupled to functionally important regions of wild-type CFTR.

We used an emerging computational approach named dynamical-nonequilibrium molecular dynamics (D-NEMD) simulations[3]. This approach combines molecular dynamics (MD) simulations under equilibrium and nonequilibrium conditions to map the evolving structural response of a protein to the removal of a ligand from its binding site. Using molecular models based on the cryo-EM structure of ivacaftor complexed with phosphorylated, ATP-bound human CFTR (PDB ID: 6O2P)[2], which lack the R domain, we conducted extensive equilibrium MD simulations (five replicate 500 ns simulations, totalling 2.5 μs) and a large set of 410 short (5 ns) nonequilibrium simulations to identify conformational changes in CFTR elicited by the removal of ivacaftor from its binding site.

Our simulations demonstrated that the structural changes induced by ivacaftor removal started in the ligand-binding pocket, namely transmembrane segments 4, 5 and 8. Subsequently, the structural changes were gradually transmitted both upwards towards the extracellular vestibule of the CFTR pore and downwards through neighbouring transmembrane segments, ultimately reaching the ATP-binding sites located at the dimer interface of the nucleotide-binding domains.

Our work reveals how ivacaftor-induced structural changes are propagated from the ligand-binding site to the ATP-binding sites and the channel pore. We will use this approach to understand how ivacaftor restores function to CFTR proteins with gating mutations to inform the rational development of structure-guided therapies for cystic fibrosis.

Supported by the Cystic Fibrosis Trust. KJS was the recipient of an ECFS / CF Europe Post-Doctoral Research Fellowship.

References:

1. Yeh et al. J Gen Physiol. 2019; 151:912-928.

2. Liu et al. Science. 2019; 364:1184-1188.

3. Oliveira et al. Eur Phys J B. 2021; 94:144.

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S6.6 Elexacaftor/Tezacaftor/Ivacaftor triple therapy corrects function of CFTR rare variants

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Objectives: In Europe the triple combination Elexacaftor/Tezacaftor/Ivacaftor (ETI) is accessible only for patients carrying p.Phe508del (F508del) mutation on at least one allele, while the Food and Drug Administration (FDA) labelled ETI for patients carrying 177 additional CFTR variants. Considering that a number of severe patients carrying rare variants may benefit from ETI, a compassionate therapy program was launched in France. Those patients were invited to perform a nasal brushing to correlate the clinical efficacy of ETI with the correction of CFTR activity evaluated in vitro in Human nasal epithelial (HNE) cell cultures.

Methods: CFTR activity correction was measured by short circuit current in HNE cultures at basal state (DMSO incubation) and after ETI incubation (48h) and expressed as % of normal CFTR activity (CFTR WT) after sequential addition of Forskolin and Inh-172 (ΔIsc ETI/DMSO %WT).

Results: Eleven patients with CF carried variants eligible to ETI according to the FDA list: I601F, G85E, S492F, M1101K, R3479P, R74W, and H1085R. Twenty eight carried variants not listed by FDA: large deletions (CFTRdele19 and CFTRdele3-10;14b-16); nonsense variants (Q493X, G542X, R553X, E585X, K710X, W1063X E1104X, R1162X, W1282X); a variant anticipated to inhibit translation initiation (M1T); variants introducing a frameshift and predicted to generate a Premature Termination Codon (357delC, 1078delT, 2183AA>G, 3659delC, 4271delC); splicing mutations (711+1G>T, 1525-1G>A, 1717-1G>A, 4374+1G>A, 2789+5G>A, 4096-3C>G); missense mutations shown to generate a severely misfolded protein (I507del, N1303K, R334W, R1066C, L558S) or whose mechanisms have not been yet documented (Q552P).

ETI significantly increased CFTR activity of FDA approved CFTR variants. We point out the correction by ETI of additional non FDA variants, including N1303K, R334W, R1066C, Q552P and terminal splicing variants (4374+1G>A, 4096-3C>G). Correction of $Isc_{ETI/DMSO}$ %WT was significantly correlated to the change in ppFEV 1 ($p < 0.0001$), and of the sweat test ($p < 0.0001$). G85E, R74W and Q552P were rescued more efficiently by other CFTR modulator combination than by ETI.

Conclusion: Primary nasal epithelial cells hold promise for expanding CFTR modulator treatment in patients carrying rare mutants. Additional variants than those listed by FDA should be discussed for ETI indication.

01 April — 11:00–12:45

Symposium 7 – Gene-based molecular approaches

S7.1 New approach to treat all CF patients with an antisense oligonucleotide

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The discovery of the Cystic Fibrosis Transmembrane conductance Regulator (CFTR) gene and protein was a major milestone in understanding and treating cystic fibrosis (CF), including developing drugs targeting the defective protein. The introduction of CFTR modulators has substantially altered the disease course in patients with drug-sensitive mutations. Still, these drugs are ineffective in some cases, particularly in patients with class I mutations, and thus remain with an unmet need. In our project, we developed an antisense oligonucleotide (TMEM16a ASO) that targets the alternative chloride channel TMEM16A (Anoctamin-1) to compensate for the defective CFTR regardless of the patient's mutations. Our oligonucleotide will target TMEM16a RNA through competitive inhibition and prevent its inhibition by microRNA-9 (Sonneville et al., Nat Comm, 2017). This study evaluated the specificity, toxicity, and effects of TMEM16a ASO in different CF models, including cell lines, primary cells, and mice.

In all F508del models tested, we have demonstrated by direct and indirect approaches that the effects of ASO TMEM16A are very specific to the target, with an absence of toxicity at different concentrations. For example, the specificity of TMEM16A ASO to TMEM16A was demonstrated when it did not affect cells pre-treated with TMEM16A inhibitor (Ani9).

Concerning the efficacy, we demonstrated in the different CF cells that the oligonucleotide could activate TMEM16a chloride efflux. This aspect was tested on cell lines by the YFP-H148Q halide sensor method and confirmed by Ussing chamber analysis by treating well-differentiated ALI cultures. More, we have shown an increase in mucociliary clearance in these CF-differentiated ALI cultures. This increase was similar to non-CF human differentiated primary cells treated with TMEM16A ASO. These results were confirmed in CF class I mutation cells and compared to Vertex drugs. Furthermore, combining TMEM16A ASO treatment with CFTR modulators also demonstrated an additive effect, suggesting the potential of a combination therapy approach. Finally, in F508del mice, we showed that chronic treatment increased weight gain and survival from 36 days to nearly 200 days and improved different dysregulated parameters.

Our results indicate that the TMEM16A ASO strategy could effectively treat all individuals with CF, including class I patients, and would warrant further investigation in clinical trials.

S7.2 RNA-based therapies for Cystic Fibrosis

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New therapies are required for the treatment of CF caused by CFTR pathogenic variants not treatable currently by modulators. Gene therapy with CFTR cDNA has been tested with viral vectors (AAV and adenovirus), and with several non-viral, lipid-based vectors, delivering plasmid DNA (pDNA) but neither approach has led to a clinically effective therapy so far, due to lack of sufficient delivery and expression. CFTR messenger RNA prepared by *in vitro* transcription (IVT) offers the opportunity for improved *in vivo* transfection efficiency compared to pDNA.

Messenger RNA, delivered by intramuscular injection of lipid nanoparticle (LNP) vectors, has been used safely in millions of people worldwide in COVID-19 vaccines. The mRNA provides a template for the cell to synthesise the encoded viral spike protein, but this approach also offers the opportunity to deliver templates for protein replacement therapy in recessively-inherited genetic diseases, such as CF. Messenger RNA provides much higher transfected expression levels than pDNA as the intracellular mRNA is translated in the cytoplasm whereas pDNA must first reach the nucleus.

Messenger RNA synthesis by IVT requires a linearised DNA template containing the coding sequence, 5'- and 3'-untranslated regions (UTRs), and 5'-poly adenylation site (polyA), while activity can be enhanced by the use of chemically-modified bases, especially those replacing uridine, in the IVT reaction, which also reduces the interferon-b inflammatory response in vivo.

The limitations of mRNA as a potential genetic therapy are its transient nature requiring repeated delivery at regular intervals, poor cellular uptake through the cells hydrophobic lipid bilayer, and susceptibility to environmental nucleases. Thus, mRNA delivery requires formulation with agents such as lipids for nuclease protection and cellular uptake. This requires nanoparticles capable of overcoming the challenge of delivery to the appropriate cell types such as mucociliary clearance.

This presentation will describe the development of a CFTR mRNA therapy for cystic fibrosis and a nanoparticle formulation for its delivery to the airway.

S7.3 Mutation-tailored base and prime editing approaches allow re-writing CFTR to treat cystic fibrosis

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Considering the more than 2000 variants reported in the *CFTR* gene, of which 401 confirmed causing CF (CFTR2.org), the most straightforward way seemingly would be to develop a single gene therapy agent for all CF-causing mutations. Several such strategies are currently in clinical testing with promising first results. The advent of highly versatile gene editing tools, such as CRISPR-Cas9 and its derivatives base and prime editing, has opened up a new era for treating genetic diseases by allowing targeted genome modifications. Importantly, these methodologies allow to preserve endogenous gene expression and regulation, in contrast to gene addition, and thus avoid ectopic CFTR expression as only in cells naturally expressing CFTR, the edit will become visible on the protein level.

CRISPR-Cas gene editing has found its way into the CF field and successful demonstrations on the correction of a handful of *CFTR* mutations so far in preclinical models, underscore its great potential for further translational validation. Base and prime editing are two of the most recent additions in the CRISPR toolbox. They both have the advantage of not inducing double stranded DNA breaks, thereby reducing substantially the level of unintended DNA modifications on- and off-target. Generally speaking, the base editor up till now has conferred higher editing outcomes of targeted *CFTR* corrections than the prime editor, although only a few studies so far have investigated the prime editor's potential in a CF context. Base editing allows very precise base conversions within a given editing window. While unwanted bystander edits can occur if multiple target bases are present within this window, the use of novel base editor variants in combination with different delivery modalities allows to circumvent some of these unintended edits (Amistadi et al., Mol Ther, 2023). Prime editing is perhaps the most versatile gene editor of all due to its ability to rewrite at least in theory, up to 93% of CF-causing mutations (Maule, Ensinck, *et al*., Rewriting CFTR to cure CF, PMBTS, 2020).

This talk will cover the latest updates on in particular base and prime editing examples, both from published work as well as some of our own work. It will show proposed workflows for streamlined design and testing in preclinical models with increasing complexity, including patient-derived cell models. Precision and safety are key when applying gene editing as a potential therapy, so also these aspects will be highlighted.

We would like to acknowledge the funding agencies for their support of our gene editing work for CF: the Belgian Cystic Fibrosis Association, King Baudouin Foundation Fund Forton and Emily's Entourage.

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S7.4 A precise adenine base editor corrects W1282X-CFTR without bystander effects

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W1282X (c.3846G>A) is the sixth most common cystic fibrosis (CF)-causing variant. This nonsense mutation generates a premature termination codon, which leads to transcript degradation by nonsense-mediated decay, and, consequently, no full-length functional CFTR protein is produced. Even though four CFTR modulators have been approved for clinical use, people with CF (PwCF) carrying nonsense mutations do not benefit from any of these targeted therapies. Adenine base editing (ABE) has attracted interest as it can correct certain nonsense mutations without creating a double-stranded break in DNA, being thus potentially safer for therapeutic use than the traditional approach using CRISPR/Cas9 nuclease. ABE uses a fusion of a Cas9 nickase and a synthetic TadA enzyme modified to convert any adenine (A) into guanine (G) in an editing window spanning nucleotides 4 to 7 of the spacer. Several research groups including ours have previously reported efficient A>G editing at position A6 which corrects the W1282X variant by ABE [1]. However, a high level of bystander editing was observed at the adjacent adenines, particularly A7.

Following previous observations that splitting the Cas9 protein into two halves would minimize the duration of nuclease activity and reduce off-target/bystander effects, we designed two new fusion proteins - NLS-FKBP12-Cas9(C)-NLS and TadA-NES-Cas9(N)-FRB - and developed a novel ABE (hereinafter split-ABE), which should only dimerise and function in the presence of rapamycin. To test the split-ABE, we generated a W1282X version of the BCi-NS1.1 cell line, an immortalized line that can differentiate into all known lung cell types. These cells were electroporated with the split-ABE or SpRY-ABE (non-split control) plasmids and treated with rapamycin or DMSO (vehicle control). The DNA was extracted from cells 72h after treatment and the target region was amplified by PCR. The editing efficiency was measured by next-generation sequencing (Amplicon-EZ, Azenta).

Using the control SpRY-ABE we detected on-target editing at A6 in 5.1% of alleles but with a high level of bystander editing at position A7 of 7%. Whilst on-target editing with the split-ABE in the presence of 200 nM rapamycin was only detected in 2.1% of alleles, almost no bystander editing at A7 was observed (≤0.5%).

In summary, we showed the development of an improved version of the already existing ABE, which can successfully correct W1282X-CFTR while substantially reducing bystander edits. The fact that the split-ABE can be regulated by a small molecule drug gives us temporal control of the editing. Currently, we are developing a system for cell type-specific expression of the split-ABE, which will allow us to have spatial control of editing. The longer-term goal is to understand which cell types, and how many of them, need to be corrected to restore CFTR function to therapeutic levels using our BCi W1282X-CFTR model.

[1] Mention K et al., 2023 (Submitted to Hum. Mol. Genet.)

Work supported by grant HARRIS21G0 from CFF and centre grants UIDB/04046/2020 and UIDP/04046/2020 from FCT, Portugal (to BioISI).

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S7.5 Targeted repair of cystic fibrosis mutations across an entire exon using Cas9 and a single guide RNA and donor template

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Precision gene editing using CRISPR is a promising therapeutic approach to treat genetic disorders, such as cystic fibrosis (CF). We, and others, have demonstrated single CF-causing mutations can be corrected via homology-directed repair (HDR) using Cas9/gRNA and a DNA donor template[1]. In genetic disorders caused by multiple mutations, such as CF, it would be beneficial to correct several different mutations located in the same exon with a single donor template/gRNA combination. However, CRISPR HDR strategies are limited by the efficiency of precision repair beyond a very short distance from the Cas9/gRNA target site.

The 95 bp exon 12 of the CFTR gene contains 18 different CF-causing variants, including the second most common CF mutation, G542X, and the second most common splice variant, c.1585-1G>A. Even with the short 39 bp distance between these two mutations, it is currently not feasible to repair both mutations with a single donor template/gRNA combination by HDR. This is because donor templates must contain regions of homology with the target DNA to facilitate the formation of a so-called D-loop in order to introduce site-specific changes by HDR. However, homologous sequences within the donor template can also induce D-loop collapse, reducing the length of the HDR editing window[2]. Here, we aim to circumvent D-loop collapse through introduction of regions of reduced-homology within the donor template repair track, but still encoding the same amino acid sequence. Using this approach, we aim to generate a single donor template and gRNA capable of repairing all CF-causing mutations across exon 12.

To demonstrate extension of the HDR editing window, we are initially focusing on developing a single gRNA and donor template to correct three exon 12 mutations, namely G542X, located within exon 12, and two splice mutations at the 5' and 3' exon-intron boundaries, c.1585-1G>A and c.1679+1G>A. To this end, we have designed and tested several gRNAs targeted to intronic regions within 50 bp of exon 12. Suitable gRNAs were identified based on indel frequency as assessed by amplification and sequencing of the target region. As a CFTR model, we are using HEK293 Flp-In cells containing the expression mini-gene (EMG) CFTR with the respective variant. To generate these cell lines, we first altered the CFTR wild-type cDNA EMG construct to incorporate exon 12 flanking introns using gBlocks and restriction digestion cloning. Site-directed mutagenesis of the resulting construct produced mutant EMG constructs which were then co-transfected with Flp recombinase to generate stable mutant CFTR cell lines. The efficiency of HDR extension with the selected gRNAs and different modified donor templates will be tested in these models to establish the viability of the editing approach. Thereafter, the three exon 12 mutations will be introduced separately into the BCi-NS1.1 human airway epithelial cell line to create physiologically relevant models of CFTR, thereby facilitating functional assessment of correction mediated by gRNAs and modified donor templates.

1. Santos et al. (2022) *J Cyst Fibros* 21: 181-187

2. Byrne et al. (2015) *Nucleic Acids Res* 43: e21

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S7.6 CFTR functional rescue after delivery of nebulized LNP/mRNA to primary human bronchial epithelial cells derived from patients with different CFTR genotypes

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Cystic fibrosis (CF) is an inherited disease caused by mutations in the *CFTR* gene that encodes a chloride channel, CFTR, located on the apical plasma membrane of specialized epithelial cells. CF causes grave damage to the lungs, digestive system, and other organs, with defective pulmonary mucociliary clearance (MCC) being one of the main causes of morbidity and mortality in CF patients. The approval of small-molecule CFTR modulators such as Trikafta significantly improved the quality of life for most CF patients with access to these drugs. However, a significant fraction of the CF patient population is not amenable to the currently approved CFTR modulators. Therefore, the search for universally applicable therapies that promote CFTR function and MCC remains a goal. To address this challenge, ReCode Therapeutics is advancing an mRNA-based treatment to restore CFTR function using optimized CFTR mRNA encapsulated in proprietary lipid nanoparticles (SORT^{TM} LNPs) delivered as an inhaled aerosol directly to the target epithelial cells of the conducting airways.

Initially, we identified a CFTR mRNA sequence and composition with improved stability that is more efficiently translated into functional CFTR protein when compared to the wildtype sequence**.** Subsequently, we formulated the optimized CFTR mRNA with different SORT^{TM} LNPs. CF patient-derived, differentiated human bronchial epithelial (hBE) cells grown at an air-liquid interface were then treated with aerosolized LNP-formulated mRNA using a commercially available mesh nebulizer. Forskolin-induced Cl^- currents measured by transepithelial current clamp (TECC24) recording showed significant CFTR functional rescue. Optimization of the lead SORT^{TM} LNP candidates led to further increases in the Cl^- current observed. Analysis of protein expression by western blot (WB) and immunofluorescence (IF) confirmed the successful delivery of mRNA and expression of CFTR protein, including translation in pulmonary ionocytes and secretory cells. Interestingly, when we investigated six different CF F508del/F508del donors the level of CFTR functional rescue varied. Two main groups were observed, one with significant CFTR functional improvement and a second group with a minimal increase in function. The latter was subjected to more extensive washing of accumulated mucus on the day prior to nebulization which increased the Cl^- flux observed, suggesting an effect of the mucus layer on transfection efficiency. We are currently investigating the ability of the LNP/CFTR mRNA to efficiently penetrate the mucus/ periciliary layer/glycocalyx (PCL-G) in CF patient-derived hBEs.

Our results demonstrate the capability of the ReCode SORT^{TM} LNPs to deliver LNP-formulated CFTR mRNA as an aerosol and increase CFTR function in well-differentiated CF hBE cultures. These preclinical data support further investigation and provide a practical approach to address a significant fraction of the patient population that does not benefit from current CFTR modulator therapy.

01 April — 14:15–16:00

Symposium 8 – Alternative targets for CF

S8.1 Pharmacological therapies for PTC mutations

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The development of therapies for cystic fibrosis (CF) patients with mutations insensitive to present pharmacological CFTR modulators is an urgent unmet need. Many patients carry premature termination codons (PTCs) as CF-causing mutations. PTCs cause production of a truncated CFTR protein whose residual function depends on the position of the mutation in the amino sequence. PTCs localized at the terminal part of CFTR coding sequence may allow the synthesis of a protein amenable to treatment with CFTR corrector and potentiators. However, this approach is limited by the nonsense-mediated RNA decay (NMD) that markedly reduces protein synthesis. We recently found that W1282X-CFTR can be rescued, also in primary bronchial epithelial cells, by a combination of SMG1i, an NMD inhibitor, plus VX-809/VX-445 correctors. We started the screening of a 9,000 compounds chemical library with a functional assay to identify novel NMD inhibitors and other pharmacological agents suitable for PTCs. So far, the screening has led to the identification of various compounds that significantly rescue anion transport in cells expressing W1282X-CFTR.

As an alternative approach for undruggable CF mutations, we are considering the possibility to bypass the CF basic defect by targeting other epithelial proteins. For this purpose, we have investigated the effect of inflammatory stimuli on human bronchial epithelia in vitro. Treatment of epithelia with IL-17A plus TNF-α, two cytokines with a relevant role in CF lung disease, causes a marked change in gene expression leading to enhanced activity of various proteins involved in ion transport (SLC26A4, ATP12A, ENaC). The net effect of cytokine treatment is the generation of a highly viscous and dehydrated epithelial surface. The switch to a more fluid state in CF epithelia could be facilitated by pharmacological modulation of alternative ion channels/transporters. We found that pharmacological inhibition of SLC26A4, an electroneutral anion exchanger, causes a significant decrease in the viscosity of epithelial surface, which may be beneficial to restore mucociliary transport.

This work was supported by CFF (GALIET22I0) and Fondazione Italiana per la Ricerca sulla Fibrosi Cistica (FFC #9/2022).

S8.2 The chloride versus bicarbonate dilemma in airway muco-obstruction. Lessons from the basolateral side.

Vinciane Saint-Criq[1], Anita Guequen[2,3], Sandra Villanueva[2], Tábata Apablaza[2], Livia Delpiano[4], Michael A Gray[4], Carlos A. Flores[2,5]

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Bicarbonate is critical for correct mucin deployment and there is increasing interest in understanding its role in airway physiology, particularly in the initiation of lung disease in children affected by cystic fibrosis, in the absence of detectable bacterial infection. The current model of anion secretion in mammalian airways consists of CFTR and TMEM16A as apical anion exit channels, with limited capacity for bicarbonate transport compared to chloride. However, both channels can couple to SLC26A4 anion exchanger to maximise bicarbonate secretion. Nevertheless, current models lack any details about the identity of the basolateral protein(s) responsible for bicarbonate uptake into airway epithelial cells. We have found that the SLC4A4 Na+-bicarbonate co-transporter mediates bicarbonate uptake in the basolateral membrane of both human and mouse airway epithelium and it's genetic silencing or inhibition produces a decrease in bicarbonate secretion, airway surface liquid pH, mucocilairy clearance velocity and mucus accumulation, resembling cystic fibrosis disease. Evidence will be given that the Slc4a4-/- might be used to test mucus transport improvements in low bicarbonate conditions.

Funded by FONDECYT 1221257

S8.3 Development of an hiPSC-based CF lung disease model to study TMEM16A as an alternative therapeutic target in Cystic Fibrosis

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Cystic Fibrosis (CF) is known as the most frequent rare disorder and is caused by mutations of the chloride channel CFTR. CF results in a severe lung phenotype by mucus accumulation, chronic infection and inflammation within the airways. Previous studies suggested that the co-expressed calcium (Ca2+)-activated Cl- channel known as transmembrane member 16A (TMEM16A) could serve as a novel therapeutic target to treat CF lung disease. Although, the precise function of TMEM16A remains unresolved, it has been hypothesised that TMEM16A should either be stimulated to compensate the CFTR malfunction and to cause rehydration of the airway mucus, or that TMEM16A should be inhibited to reduce the mucus release within the airways. However, currently available disease models do not enable to conclusively elucidate the function of TMEM16A as a modifier of the CF lung phenotype.

In this study, human induced pluripotent stem cells (hiPSCs) were utilised as an emerging model system to study CF lung disease as well as TMEM16A function. Therefore, healthy (WT) and CF patient-specific (CF) hiPSCs were differentiated into airway epithelial cells in air-liquid-interface cultures (iALI cultures). It could be confirmed that iALI cultures exhibit important molecular and functional features of primary derived airway epithelial cells (pALI cultures). In particular, CF iALI cultures demonstrated an evident CF disease phenotype that is characterised by a reduced CFTR protein expression, impaired CFTR-dependent transepithelial Cl- conductance and a reduced cilia beating frequency. Notably, the CF disease phenotype could be modulated and partially rescued by application of CFTR modulator drugs.

Furthermore, TMEM16A knockout (T16KO) and TMEM16A(abc) overexpressing (T16abcOX) cell lines were generated from WT, CF and gene corrected CF patient-specific (Corr) hiPSCs to study TMEM16A in a so far unique approach. Initial experiments were conducted to assess the TMEM16A-dependent transepithelial Cl- conductance and confirmed an increased Cl- conductance in CF-T16abcOX iALI cultures. Going forward, TMEM16A-dependent effects on the mucociliary function will be explored in more detail by characterisations of the cilia beating frequency, mucociliary transport and especially of the airway surface liquid (ASL) properties as well as the mucus release. In summary, it was demonstrated that iALI cultures severe as valid disease model to study the CF lung disease. Incorporation of genome edited T16KO and T16abcOX hiPSCs enable a novel approach to further evaluate TMEM16A as a modifier of the CF lung phenotype. Our platform demonstrates the versatility of hiPSC-based disease models to investigate alternative targets for CF. In future they might enable to investigate multi-organic aspects of CF as well, by differentiation of hiPSCs into airway epithelial cells and other CF-affected linages.

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S8.4 Identification of pharmacological modulators of the calcium-signaling cascade

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Background: Pharmacological modulation of epithelial ion transport mediated by alternative ion channels could bypass the CFTR defect in cystic fibrosis (CF). For this purpose, we considered the stimulation of calcium-dependent chloride secretion mediated by TMEM16A (ANO1) protein as a possible strategy.

Aims: To identify pharmacological activators/potentiators of calcium-dependent chloride secretion in airway epithelial cells.

Methods: Using FRT cells expressing TMEM16A, we screened a maximally diverse small-molecule chemical library (11,300 compounds) with a functional assay based on the halide-sensitive yellow fluorescent protein (HS-YFP). Primary active compounds were tested with a panel of secondary assays to understand the mechanism of action. Such assays included evaluation of intracellular Ca^{2+} mobilization triggered by extracellular agonists or by caged-IP_3 photolysis, analysis of PIP_2 breakdown by phospholipase C, and ion channel recordings on intracellular membranes.

Results: Three compounds were identified to consistently potentiate TMEM16A activity following a purinergic stimulus. Analysis of the mechanism of action revealed that these compounds do not directly act on TMEM16A but modulate calcium signaling at different levels. One compound appears as a potentiator of the purinergic P2RY2 receptor. The second compound is instead a selective potentiator of the inositol triphosphate receptor type 1 (ITPR1). The third compound is a possible modulator of phospholipase C. All compounds were effective in enhancing calcium-dependent chloride secretion in airway epithelial cells from CF patients (nearly 2.5fold increase with respect to control). Moreover, the first and third compound showed an inhibitory effect on ENaC current that may result from enhanced PIP_2 breakdown.

Conclusions: Compounds identified in our study will be useful as tools of research to investigate the regulatory mechanisms that control ion transport in airway epithelia. They can also be considered for the development of therapeutic agents to improve mucociliary clearance in CF.

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P82

S8.5 TAVT-135, a novel chloride ion transporter for the pan-genotypic treatment of cystic fibrosis: electrophysiological and mucus-hydration properties

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Objectives: The treatment of cystic fibrosis (CF) has been transformed by the introduction of modulators of the CF transmembrane conductance regulator (CFTR). However, approximately 10% of patients with CF have ineligible genotypes. TAVT-135, a novel small molecule-peptide conjugate composed of a chloride ion-binding moiety and a cell-penetrating peptide (CPP), is being investigated as a potential treatment for CF, regardless of CFTR mutational status. Here, we report the results of a series of studies that were performed to characterize the electrophysiological and mucus-hydration properties of TAVT-135.

Methods: The effect of TAVT-135 (10 μM) or its separate functional components on intra- to extracellular chloride ion transport was evaluated in *Xenopus laevis* oocytes using a two-electrode voltage-clamp technique.

Anion efflux was then evaluated by means of a modified Ussing chamber system in human bronchial epithelial (HBE) cells harboring mutations for non-functional CFTR. TAVT-135 (0.01, 0.1, 1, 10, 25, and 50 μM) was acutely added to the apical side of the cultures. Short circuit current (I_{SC}) following amiloride-induced inhibition of the epithelial sodium channel (ENaC) was measured, along with transepithelial electrical resistance (TEER).

Mucus hydration in HBE cells with CFTR mutations was assessed by measuring the height of the airway surface liquid (ASL) and the periciliary layer (PCL). Following chronic exposure to TAVT-135 (1, 10 and 100 μM) for 48 hours, ASL and PCL were visualized with apical application of FITC-dextran; z-stack images were obtained by confocal microscopy to determine heights (μm).

Results: In *X. laevis* oocytes, TAVT-135 induced a rapid chloride efflux, demonstrating chloride transport from the intracellular to the extracellular space. The CPP alone resulted in a slight change in chloride current, but the magnitude of response was typically around 5% of TAVT-135. The unconjugated chloride-binding component did not have any detectable effect on chloride current.

In HBE cells, there was a statistically significant, dose-dependent increase in I_{SC} following the acute application of TAVT-135 at concentrations ≥1μM, demonstrating anion efflux. Following 5 minutes of exposure, TEER was maintained at TAVT-135 concentrations ≤1μM and decreased at concentrations ≥10μM.

TAVT-135 (10 and 100 μM) statistically significantly increased ASL and PCL height in HBE cells with CFTR mutations after 48 hours of incubation compared with the untreated control.

Conclusions: In these *in vitro* experiments, TAVT-135 rapidly induced intracellular chloride transport across plasma membranes without negatively impacting the epithelial barrier. TAVT-135 also promoted ASL hydration and increased PCL height, which may suggest a mucociliary clearance effect *in vivo*. These data support that TAVT-135 has the potential to address significant unmet needs in patients with CF, including those who are ineligible for, or not responding to, CFTR modulators.

01 April — 16:30–17:30

Closing Keynote lecture

In Vivo gene editing

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Cystic fibrosis (CF), an autosomal recessive disorder caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene, is a life-shortening multisystem chronic illness. CFTR is an anion channel key to ion and fluid balance in epithelial tissues. CF patients experience multiorgan dysfunction in the respiratory, gastrointestinal, and reproductive tracts as well as eccrine glands. In addition, patients experience complications such as CF-related diabetes and liver disease. While there are hundreds of CF-causing mutations, the most common mutation is F508del(1521_1523del) results in improper trafficking and function of CFTR. The recent advancement of modulator therapies designed to mitigate effects of many CFTR mutations including the F508del mutation holds great promise, but these regimens require expensive and continuous treatment. Moreover, they are not helpful in approximately 10% of patients with rare mutations who are not candidates for modulator therapies. An alternative therapeutic approach for patients with CF is precise correction of these genomic mutations.

Efforts to precisely correct genomic mutations that underlie inherited disorders such as CF have advanced with the emergence and refinement of genome editing technologies such as nuclease-based platforms which include zinc finger nucleases (ZFNs), TALENs, as well as CRISPR/Cas systems including base and prime editors, and oligo/polynucleotide strategies such as triplex-forming oligonucleotides (TFOs). Recent *in vivo* gene editing therapies have focused on targeted correction of the CFTR gene in the airway epithelium. However, CF is a systemic disease with multiple affected organs that could potentially benefit from gene correction therapies.

Our recent focus has been to correct the multiple organ dysfunction of CF-causing mutations using systemic delivery of peptide nucleic acid gene editing technology mediated by biocompatible polymeric nanoparticles. We have developed a non-nuclease-based approach to gene editing by utilizing endogenous DNA repair stimulated by the binding of peptide nucleic acids (PNAs) to genomic DNA to create a PNA/DNA/PNA triplex structure via both Watson-Crick (WC) and Hoogsteen H-bonding with displacement of the non-bound DNA strand. PNAs have a peptide backbone but undergo base pairing with DNA and RNA. Although they lack intrinsic nuclease activity triplex PNA structures can initiate an endogenous DNA repair response mediated by high fidelity nucleotide excision repair (NER) and homology-dependent repair (HR) pathways. When PNAs are introduced with a single-stranded "donor" DNA containing the desired sequence modification, site-specific modification of the genome occurs. Using this platform, we have been able to demonstrate their use both *in vitro* in primary cells grown in a physiologically relevant air-liquid interface (ALI) culture model and *in vivo* in mice homozygous for the F508del mutation. *In vivo* treatment resulted in a partial gain of CFTR function in epithelia as measured by *in situ* potential differences and Ussing chamber assays and correction of CFTR in both airway and GI tissues with no off-target effects above background. Our data suggest that systemic delivery of PNA NPs designed to correct CF-causing mutations is a viable option to ameliorate the disease in multiple affected organs.

P1

Extending the success of Trikafta to rare mutations - insights from CFTR structure and modulator binding

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Efficient genotype specific CFTR modulators, ELX/TEZ/IVA, are approved, offering therapeutic opportunities for most CF patients. But they have not been approved for many rare mutations. The use of ex-vivo models, such as rectal organoids, enables testing CFTR modulators in individuals not included in clinical trials.

We established patient-derived organoids to test for modulator response of the rare mutation Q1100P. This mutation is not reported in CFTR2, although there are scattered mentions in the literature of patients carrying this allele. We obtained biopsies from 3 patients heterozygous for the mutation. Their second allele was either a stop mutation (C225X that is not expected to be responsive to ELX/TEZ/IVA) or an unreported missense mutation (K193E). The basal CFTR activity as measured by forskolin swelling assay (FIS) was minimal. We assessed function after treatment with each modulator separately or in combinations.

In Q1100P/C225X organoids patient, VX-661 alone had no effect. CFTR function was partially restored with VX-445 and was further increased in combination with VX-661, without additional benefit from VX-770. The Q1100P mutation causes the introduction of a proline residue instead of the WT glutamine, expected to disrupt the alpha-helix structure of TM11. A recently published structure of CFTR in complex with VX-445 (1) shows binding of the modulator to two amino acids flanking Q1100P (1098 and 1102). This raises the possibility that VX-445 binding corrects the structural defect introduced by the Q1100P mutation, leading to functional correction of the protein. VX-661 further increased the correction of CFTR function by VX-445 in these organoids, consistent with other studies showing a synergistic effect of VX-661 and VX-445 in improving the CFTR F508del folding and function (1).

In Q1100P/K163E organoids, CFTR function was partially restored by treatment with VX-661 alone, and significantly restored by treatment with VX-445 alone. Interestingly, no additive effect above that of VX-445 was achieved with the combination of VX-661+VX-445. No additional benefit was seen from addition of VX-770. In these organoids, function restoration might arise from both alleles. K163E is a missense mutation in a conserved lysine residue participating in ICL1-NBD1 interface, which is crucial for CFTR folding and corrected by VX-661 (2). Interestingly, VX-661 in these organoids did not contribute to the high response to VX-445. Further studies with organoids from a patient homozygous for K163E are ongoing and will shed light on the molecular basis of the modulator responses.

Based on our results, treatment with Trikafta was approved by the health provider for two Q1100P/K163E patients. Improvement in clinical parameters was immediate. FEV1 improved by 15% in one patient and 21% in the other, accompanied by a decrease in LCI (by 5.6 and 5 points) and decreased sweat chloride (9 and 30 mmol/L) in the first and second patients, respectively.

Altogether, we show how knowledge of the CFTR structure and modulator binding sites can guide in prioritizing the analysis of rare mutations to existing treatments in patient-derived organoids.

References:

1. Fiedorczuk K, Chen J., Science 2022;378.

2. Kleizen, B. et al., J. Mol. Biol 2021.

P2

Microfluidic platform for water transport measurements across WT and CF bronchial epithelium

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Airway epithelium is constantly exposed to inhaled particulate matter (PM) and pathogens. Lumen side of airways is lined by Airway Surface Liquid (ASL) which consists of a low viscosity periciliary liquid (PCL) and the mucus layer (ML). The composition, volume and pH of the ASL are key physiological parameters that are related to airway hydration, reactivity and antimicrobial activity and are very finely regulated. Epithelial ion transport regulates the volume and composition of the ASL, mainly through modulation of Na^+ absorption and Cl^- secretion. The defect of the epithelial transport leads to serious diseases such as cystic fibrosis – life limiting disease characterized by abnormal ASL volume and/or composition.

Previous studies of ion transport across the 16HBE14o- cell monolayers in ionic gradients showed that the transport of ions to the chamber with lower concentrations of Cl^- and Na^+ is of one order of magnitude higher than the decrement on the higher concentration side. We found that the imbalance of Na^+ or Cl^- concentration on both sides of the epithelium causes the flux of isosmotic NaCl solution across the epithelial layer and thus decreasing the ion gradient. To test our findings experimentally, we built a special microfluidic system allowing for the direct measurements of water transport across epithelial cell monolayers. To test the role of CFTR channel in ASL hydration, we performed the experiments on two different cell lines: 16HBE14o- (WT) and CFBE41o- (CF).

Our results show that the water flows through the epithelium exposed to ionic gradients, confirming our findings from multi-ion transport measurements. The difference between water transport across WT and CF cell monolayers was observed. The transport rate was higher when low Cl^- solution was introduced on the apical side of monolayer indicating the role of CFTR in this process. In Na^+ gradients, higher water transport in CF cells were seen when high Na^+ solution was introduced on apical side demonstrating the role of ENaC channel in epithelial water absorbtion. Interestingly, our microfluidic system was able to detect the water fluxes in symmetric solutions. The transport across WT cells was higher than across CF cells. Forskolin increased the water flux across WT and CF cells. Addition of Inh-172 reduced the volume of water transported across WT cells however did not block it completely. Total inhibition of water transport after addition of Inh-172 was observed in CF cells. Our results show that CFTR plays a role in ASL hydration, however there are also other ion transporting proteins involved in this process.

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P3

Tissue-specific *cis*-regulation of the *CFTR* gene

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The *Cystic Fibrosis Transmembrane conductance Regulator* (*CFTR*) gene was discovered in 1989 and more than 2000 mutations have been identified. However, it is still challenging to diagnose patients with cystic fibrosis and also *CFTR*-related disorders, such as congenital bilateral agenesis of the vas deferens (CBAVD) or pancreatitis. Indeed, some patients have incomplete genotypes or present extreme phenotypes. In order to gain insight, it is important to understand the distinction between cystic fibrosis and the borderline forms. To explain those complex cases, the project aims to identify potential dysregulation of the *CFTR* gene expression due to alterations of *cis*-regulatory elements (CREs).

Regulation of the *CFTR* gene is explore in a cell-specific manner. Chromatin study techniques are implemented in three cellular models, intestinal, pancreatic and epididymal cells. These are the main organs involved in diseases involving the *CFTR* gene, after the lungs, but which have already been well documented. The 4C (Circular Chromosome Conformation Capture) technique is used to obtain chromatin interaction profiles and define candidate CREs (cCREs) which are then validated by reporter gene tests. CUT &RUN (Cleavage Under Targets And Release Using Nuclease) method is used to define the H3K27ac (enhancer) and H3K27me3 (silencer) epigenetic marks as well as the CTCF (CCCTC-binding factor) marks. A second part is devoted to the detection of variants within CREs by Next Generation Sequencing and defining their implications.

4C analyses indicate multiple regions interacting with the *CFTR* promoter. Many interactions are conserved between cell type, but specific peaks are also observed. Reporter gene assays performed on cCRES confirmed the presence of enhancers as well as a potential silencer in a long-range distance. Through sequencing of CBAVD patients, eight potential regulatory variants were identified in comparison to the frequency of the European population. In order to validate their impact on the three-dimensional regulation of the *CFTR* locus, functional tests are performed.

We provide new elements which could help, in the future, to better understand the physiopathology of *CFTR* diseases. To go further, we want to define the transcription factors involved in the regulation of the *CFTR* gene. In addition, we need to use CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) technology to have an endogenous tool that can lead us to confirm the gene regulation within those three models.

Thus, this work allows us to better understand the three-dimensional organisation of the *CFTR* locus in order to improve the management of patients.

P4

A synonymous polymorphism associated with rare *CFTR* variants confers alterations to protein biogenesis, pharmacologic response, and clinical phenotype

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People with CF carry more than 250 different synonymous or “silent” single nucleotide polymorphisms (sSNPs) in *CFTR* that are often viewed as neutral for protein folding and function. We previously identified a relatively common sSNP (c.2562T>G) that inverts local translational speed at the affected codon (T854T), leading to alterations in CFTR topology and ion transport through a mechanism dependent on ribosome velocity. When c.2562T>G is present in cis, this sSNP induces subtle structural rearrangements to counteract destabilizing effects of certain rare *CFTR* variants (G551D, D579G, D614G), thereby enhancing channel function.

In the present study, we assessed impact of the c.2562T>G sSNP on CFTR biogenesis and pharmacologic sensitivity using Fischer rat thyroid cells and CF bronchial epithelia transiently expressing mutant CFTR cDNA with or without c.2562T>G. Our findings indicate this sSNP increases maturation and transepithelial ion transport mediated by rare *CFTR* variants, such as D579G and D614G, following treatment with clinically approved small molecules (e.g. VX-809, VX-770). Additionally, sSNPs engineered to alter the speed of translation at the primary disease-causing mutation (i.e. “revertants”) were found to exert strong effects on CFTR modulator-dependent rescue.

Using whole-genome sequencing data from the CF Genome Project, we next interrogated prevalence and clinical relevance of the c.2562T>G sSNP across 5,058 CF subjects in the United States. Approximately 22% of this population harbors one or two copies of c.2562T>G. This sSNP is predominantly associated with rare *CFTR* variants, as evidenced by a frequency of 0.28% among F508del alleles. Interestingly, patients with the *CFTR* genotype, 3849+10kbC>T / F508del, exhibit substantially different sweat chloride levels depending on the presence or absence of c.2562T>G, which is presumed to reside on the 3849+10kbC>T allele. For these individuals, mean sweat chloride is significantly lower among people who encode c.2562T>G (52.2 mmol/L; SD ± 15.4) compared to those who do not have the sSNP (69.7 mmol/L; SD ± 16.8) ($P = 0.0344$ Mann-Whitney, ranksum).

Our results argue against neutrality of *CFTR* sSNPs during protein biogenesis, highlighting ways in which silent mutations can change local kinetics of mRNA translation and epistatically modulate outcomes of CF-causing variants. Such effects are likely to influence the spectrum of disease symptoms, represent a mechanistic contributor to genotype-phenotype relationships, and ultimately may help predict therapeutic response in precision theratyping studies.

This work is supported by the U.S. National Institutes of Health, U.S. Cystic Fibrosis Foundation, German Cystic Fibrosis Foundation – muko E.V., and Deutsche Forschungsgemeinschaft. In addition, we thank Karen Raraigh and Prof. Dr. Garry Cutting (Johns Hopkins University; Baltimore, Maryland, USA) for providing data from the CF Genome Project.

P5

Analysis of CFTR correctors mechanism of action

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Background: Pharmacological modulators of CFTR channel gating and trafficking are valuable scientific tools and therapeutic agents to treat the basic defect in cystic fibrosis (CF). In particular, correction of the molecular defects associated with F508del mutation requires combination of molecules with complementary mechanisms of action (MoA) to obtain optimal rescue. CFTR correctors, i.e. compounds that improve the folding and trafficking of F508del-CFTR, can be grouped into three classes according to synergistic/additive effects generated by corrector combinations (Veit et al., Nat Med 2018). Corrector classes may also correspond to distinct binding sites in the CFTR protein, with class 1 and class 3 involving possible interaction with membrane spanning domain 1 (MSD1) and nucleotide-binding domain 1 (NBD1, respectively).

Aims: To investigate how different correctors act on CFTR protein.

Results: We evaluated VX-809 and VX-445, as class 1 and class 3 correctors, respectively, and PP028, a novel corrector, developed at the University of Palermo, whose MoA is still undefined. The effect of combinations of such molecules was determined at the functional level with the halide-sensitive yellow fluorescent protein (HS-YFP) assay. VX-445 or PP028 elicited a large F508del-CFTR rescue when combined with VX-809. These results were confirmed in short-circuit current recordings on cultured bronchial epithelia from CF patients. A biochemical approach was used to investigate the site of action of compounds on CFTR protein. Briefly, HEK293 cells were transfected with plasmids coding for fragments of CFTR protein of increasing length and then treated for 24 h with single correctors or corrector combinations. The effect of CFTR fragment stabilization elicited by corrector treatments was evaluated by immunoblot as an increase in protein band intensity. VX-809, but not VX-445 or PP028, stabilized the MSD1 fragment (amino acids 1-367). VX-445 and PP028 were also ineffective in fragments including NBD1 (amino acids 1-633) or NBD1 plus R domain (amino acids 1-823). Instead, a significant stabilization effect was observed with VX-445 and PP028 on a CFTR fragment that also included membrane spanning domain 2, MSD2 (amino acids 1-1182).

Conclusions: In contrast to previous conclusions, indicating NBD1 as the site of action of class 3 correctors, we found evidence for MSD2 as the target for these molecules. Our results agree with a recent study (Fiedorczuk and Chen, Science 2022) indicating MSD2 as the binding site for VX-445.Our results also indicate PP028 and related compounds as class 3 correctors with a mechanism and site of action resembling that of VX-445. This information will be important for the generation of novel correctors with improved potency and efficacy.

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P6

Partial correction of F508del-CFTR trafficking and stability defects by the combination of PTI-801 with ABBV-2222 or FDL-169

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Background/Aim: The most prevalent cystic fibrosis (CF)-causing mutation -- F508del (c.1521_1523delCTT, p.Phe508del) -- impairs the folding and stability of the CFTR protein, resulting in its defective trafficking and premature degradation. Despite the significant progress in developing small-molecule correctors, the mechanism of action (MoA) of these compounds needs to be further elucidated to achieve a more robust rescue of F508del-CFTR. Here, we investigated the efficacy and MoA of PTI-801 (posenacaftor), a newly developed F508del-CFTR corrector.

Methods: The effects of PTI-801 were assessed by biochemical, immunofluorescence microscopy, and functional assays in CF bronchial epithelial (CFBE) cell lines expressing F508del-CFTR and further confirmed in 16HBE gene-edited F508del-CFTR cells. To shed light on the MoA of PTI-801, its additive effects to correctors (VX-445, VX-661, ABBV-2222, FDL-169, and Corr-4a), genetic revertants of F508del-CFTR (G550E, R1070W, and 4RK) and the traffic-null variant DD/AA were assessed.

Results: The maximal rescue of F508del-CFTR processing and function by PTI-801 was achieved at 3 μM concentration. PTI-801 was additive to low-temperature incubation in rescuing F508del-CFTR processing function (upon acute stimulation with forskolin and VX-770). PTI-801 also elicited a greater rescue of F508del-CFTR processing and function in combination with VX-661, ABBV-2222, FDL-169 (type I correctors), and Corr-4a (type II corrector), but not with VX-445 (type III corrector). Both PTI-801 and VX-445 demonstrated similar behavior in the processing of CFTR genetic revertants. They were additive to revertants G550E, R1070W, and 4RK and did not rescue DD/AA trafficking. Despite the high efficacy of PTI-801 with ABBV-2222 or FDL-169, or in a triple combination with Corr-4a, protein half-life analysis using cycloheximide revealed that these corrector combinations were unable to restore F508del-CFTR stability to WT-CFTR levels.

Conclusion: The lack of additivity of PTI-801 with VX-445 suggests that these compounds may act by a similar MoA to rescue F508del-CFTR.

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P7

Rescuing rare CFTR mutants by a mimetic peptide targeting the AKAP function of PI3Kγ

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Background and rationale: Cystic fibrosis (CF) is caused by mutations in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR), a cAMP-activated Cl- channel whose dysfunction leads to respiratory failure. The approval of CFTR modulators has opened the possibility of targeting the basic molecular defects underlying CF. Nevertheless, these molecules fail to completely rescue the activity of CFTR mutants (up to 10 and 60% of physiological values), and patients with rare mutations are not eligible for these treatments.

Hypothesis and objectives: We previously identified phosphoinositide 3-kinase γ (PI3Kγ) as a PKA-anchoring protein that tethers PKA to cAMP hydrolysing enzymes, restraining CFTR channel activation. Accordingly, we have conceived a cell-permeable mimetic peptide (PI3Kγ MP) that, by targeting PI3Kγ scaffold activity, increases sub-cortical levels of cAMP, the second messenger known to increase channel activation and stabilization. Notably, in bronchial epithelial cells the PI3Kγ MP induced cAMP elevation in the proximity of CFTR, triggering PKA-mediated phosphorylation of S737-residue and opening of CFTR. In F508del-primary bronchial epithelial cells, PI3Kγ MP doubled the effects of Trikafta® on Cl- conductance by increasing CFTR stabilization. Here, we hypothesize PI3Kγ MP as an alternative therapeutic strategy to restore the function of rare class III/IV mutants that can be responsive to cAMP stimulation. Furthermore, we will verify that the compound possesses particle size and mucus permeability suitable for lung delivery.

Materials and methods: Functional CFTR assays were performed in gold-standard preclinical models of CF, i.e. patient-derived bronchial/nasal epithelial cells and intestinal organoids treated with PI3Kγ MP, alone or with CFTR modulators. The peptide permeability and the aerodynamic properties were assessed by Parallel Artificial Membrane Permeability (PAMPA) assay and Next Generation Impactor (NGI) study, respectively.

Results: We found that, the peptide rescued the opening of the channel in intestinal organoids derived from compound heterozygotes bearing the frameshift mutation 2184insA and the R334W allele, a rare Class III mutant that is currently not eligible for modulator treatment. Measurement of short-circuit currents (I_{SC}) showed that acute application of PI3Kγ MP, but not VX-770, induced an increase in CFTR conductance when applied either alone or in association with Fsk. Intriguingly, the peptide retained the ability to partially increase I_{SC} even in the presence of a CFTR inhibitor, suggesting the ability of the compound to trigger Cl- secretion not only through a direct action on the CFTR, but also indirectly, by activating channels that enhance the electrochemical driving force. Finally, we demonstrated that, in PAMPA assay the peptide is able to cross the phospholipidic barrier in presence of CF-mimicking mucus and has aerodynamic mean dimensions suitable for inhaled delivery in humans when nebulized by mesh nebulizers, with a respirable fraction higher than 90%.

Conclusions: Overall, these results demonstrate that the peptide has optimal chemical and aerodynamic properties for lung delivery. Furthermore, these features make PI3Kγ MP a promising therapeutic strategy to directly rescue the activity of rare class III/IV mutants that can be responsive to cAMP stimulation or potentiate the effects of CFTR modulators on mutants for which these treatments have been approved.

P8

ABC-transporter CFTR folds with high fidelity through a modular, stepwise pathway

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The question how proteins fold is especially pointed for large multi-domain, multi-spanning membrane proteins with complex topologies. We have uncovered the sequence of events that encompass proper folding of CFTR in live cells, by combining kinetic radiolabeling with protease-susceptibility assays. We found that CFTR folds in two clearly distinct stages. The first, co-translational, stage involves folding of the 2 transmembrane domains TMD1 and TMD2, plus one nucleotide-binding domain, NBD1. The second stage is a simultaneous, post-translational increase in protease resistance for both TMDs and NBD2, caused by assembly of these domains onto NBD1. Our assays probe every 2–3 residues (on average) in CFTR. This in-depth analysis at amino-acid level allows detailed analysis of domain folding and importantly also the next level: assembly of the domains into native, folded CFTR. Defects and changes brought about by medicines, chaperones, or mutations also are amenable to analysis. We here show that the well-known disease-causing mutation F508del, which established cystic fibrosis as protein-folding disease, caused co-translational misfolding of NBD1 but not TMD1 nor TMD2 in stage 1, leading to absence of stage-2 folding. Corrector drugs rescued stage 2 without rescuing NBD1. Importantly, the assay can discriminate between modes of action of different correctors. Likewise, the DxD motif in NBD1 that was identified to be required for export of CFTR from the ER we found to be required already upstream of export, as CFTR mutated in this motif phenocopies F508del CFTR. The highly modular and stepwise folding process of such a large, complex protein explains the relatively high fidelity and correctability of its folding.

P9

Combination of novel potentiators with VX-770 enhances R334W-CFTR function

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Background/Aim: The R334W (c.1000C>T, p.Arg334Trp) is a rare cystic fibrosis (CF)-causing mutation for which no CFTR modulator therapy is currently approved. This mutation has minimal impact on CFTR protein trafficking but significantly reduces channel conductance that still allows for residual CFTR function. In cell lines, R334W-CFTR exhibited no to suboptimal susceptibility to the clinically approved potentiator VX-770. Recent reports have suggested that a combination of potentiators with complementary mechanisms (i.e., co-potentiators) may be a feasible strategy for mutations that do not respond well to a single potentiator. Here, we screened a collection of novel compounds to identify potentiators for R334W-CFTR. The potential utility of active compounds as co-potentiators with VX-770, genistein, and VX-445 was also exploited.

Methods: A new CF bronchial epithelial (CFBE) cell line co-expressing the halide sensitive yellow fluorescence protein (HS-YFP) and R334W-CFTR was generated to screen a collection of compounds and identify novel potentiators. The active compounds were then validated by Ussing chamber measurements, and their additive effects in combination with VX-770 (1 μM), genistein (50 μM), or VX-445 (3 μM) were exploited in this cell line and further confirmed by the forskolin-induced swelling assay of intestinal organoids (R334W/R334W genotype).

Results: Four compounds (LSO-24, LSO-25, LSO-38, and LSO-77) were active in the HS-YFP assay on a plate reader, and their ability to enhance R334W-CFTR-dependent chloride secretion was confirmed using electrophysiological measurements. Moreover, *in silico* ADME analyses demonstrated that these compounds follow obey Lipinski's rule of five for drug-like molecules and are suggested to have good bioavailability. Dose-response relationship revealed nevertheless suboptimal efficacy exerted by these compounds. VX-770 and genistein also exhibited a small potentiation of R334W-CFTR function, while VX-445 demonstrated no potentiator activity for this mutation. An additive potentiation of CFTR function was observed when the compounds LSO-24, LSO-25, LSO-38, and LSO-77 were used in combination with VX-770, but not with genistein, in both R334W-heterologously expressing cells and in intestinal organoids from an R334W homozygous CF individual.

Conclusion: The compounds LSO-24, LSO-25, LSO-38, and LSO-77 demonstrated to further potentiate R334W-CFTR function in combination with VX-770, suggesting that they act by different mechanisms. They may thus provide a valuable starting point for the design of analogs with improved CFTR potentiator activity.

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P10

Photo-Affinity Labeling (PAL) as a suited approach to investigate the mechanism of action of CFTR corrector ARN23765

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Cystic Fibrosis (CF) is a rare genetic disease characterized by deficiencies in the synthesis or function of the CF transmembrane conductance regulator (CFTR) anion channel, caused by mutations in the CFTR gene. Small-molecule compounds addressing the basic defect of the disease have been described, and are referred to as CFTR modulators.[1] Among these, our group discovered *ARN23765*, a potent F508del-CFTR corrector, which showed high potency in rescuing the function of mutant CFTR in primary human bronchial epithelial cells from F508del/F508del CF patients.[2] Despite the validated pharmacological effects, *ARN23765* mechanism of action has not yet been conclusively defined.[3] CFTR correctors can act either directly by binding to CFTR or by interacting with the protein machinery responsible for protein synthesis and maturation.

To the best of our knowledge, no data are so far available disclosing the interaction of modulators with CFTR (either wt- or F508del mutant) in a native cellular environment.

Our project aims to identify the biological target(s) and the mechanism of action of *ARN23765* by Photo-Affinity Labeling (PAL) studies in live cells.[4,5] A set of photo-affinity probes (PAPs) has been synthesized by introducing in the structure of *ARN23765* a photo-reactive moiety and a reporter/purification tag or a chemical handle suitable for conjugation to such a tag.[6] The photo-reactive moiety can be activated by UV light to generate highly reactive transient chemical species that crosslink in a covalent manner to bio-molecules in close proximity. The reporter molecule is used to detect and/or isolate probe-protein adducts for the identification of the target(s) by Western blot and/or mass spectrometry analysis.

We synthesized *ARN23765*-like photo-affinity probes that allowed capturing CFTR in wt- and F508del-CFTR-overexpressing CFBE41o- cells, demonstrating for the first time the interaction of a corrector to CFTR in live cells. Additional targets or proteins interacting with CFTR, which were identified with PAL experiments coupled to mass spectrometry techniques, are still under evaluation.

PAPs proved an efficient tool to investigate *ARN23765* mechanism of action in live cells, demonstrating that the corrector may rescue the activity of the mutant CFTR by interacting with the mutant protein itself and possibly with components of the CFTR interactome. To the best of our knowledge, this finding represents an unprecedented outcome since the interaction of a modulator with CFTR was analysed in a native biological environment. This approach allowed identifying a set of interesting proteins involved in CFTR interactome, possibly representing additional targets of *ARN23765*.

Further studies are ongoing to elucidate *ARN23765* mechanism of action and binding site, which will hopefully contribute to better characterize this corrector.

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References:

1. Lopes-Pacheco, M. et al. Exp. Opin. Drug Discov. 2021, 16, 897-913
2. Pedemonte, N.; Bertozzi, F. et al. Sci. Adv. 2020, 6, eaay9669
3. Pankow, S. et al. Nature 2015, 528, 510-516
4. Dubinsky, L. et al. Bioorg. Med. Chem. 2012, 20, 554-570
5. Smith, E. et al. Future Med. Chem. 2015, 7, 159-183
6. Hill, J.R. et al. J. Med. Chem. 2018, 61, 6945-6963

P11

Exploring the potentiating effects of VX-445 on CFTR: a functional perspective

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Cystic fibrosis is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene, which encodes an anion channel regulating transepithelial fluid movement. The most prevalent mutation, F508del, impairs folding, leading to reduced CFTR at the cell membrane and defective gating.

The standard-of-care treatment for patients carrying F508del, Trikafta (Vertex Pharmaceuticals), combines the potentiator VX-770 (ivacaftor), the corrector VX-661 (tezacaftor), and the dual-activity modulator VX-445 (elexacaftor). Initially, it was believed that VX-445 operates primarily as a corrector by stabilising CFTR's nucleotide-binding domain 1 (NBD1) (Veit et al., 2020; JCI Insight 5:e139983). However, recent studies demonstrated that it also increases CFTR open probability (Veit et al., 2021; J Cyst Fibros 20:895-898). Despite recent cryo-EM structures of F508del-CFTR bound to VX-445 (Fiedorczuk and Chen, 2022; Science 378: 284–290), the underlying mechanism of action remains to be fully understood. VX-445 also potentiates G551D- and G1244E-CFTR, variants that have mutations which affect ATP-dependent conformational changes associated with gating. In addition, its effects show synergy with VX-770 on G551D-CFTR (Veit et al., 2021; J Cyst Fibros 20:895-898).

We have found that the R1030A mutation, located in transmembrane helix 10, leads to a reduction in the potency of BPO-27, an ATP-site targeting CFTR inhibitor. R1030A also attenuated the severity of the gating impairment caused by the G551D, G1244E, S1251N, and G1349D mutations, positioned at the interface between CFTR's two NBDs. These findings are consistent with R1030A enabling non-canonical open conformations along with NBD-dimerised canonical ones. We aim to investigate the mechanism of action of VX-445 by evaluating its effects on G551D-CFTR and comparing wild-type and R1030A backgrounds.

To conduct our study, we employed a high-content image-based assay that enables the simultaneous quantification of CFTR membrane proximity and ion-channel function at the single-cell level in human embryonic kidney (HEK-293) cells. We utilised the pIRES2-mCherry-YFPCFTR plasmid to co-express CFTR, tagged at its N-terminus with the halide-sensitive yellow fluorescent protein (YFP, H148Q/I152L) and the red cytosolic fluorescent protein mCherry. The latter allows us to determine the areas and borders of cells and their membrane-proximal zones. Through image analysis, we were able to quantify the total CFTR present across cells and the mature, membrane-proximal CFTR based on the position and intensity of YFP-CFTR fluorescence. All constructs were activated via forskolin-mediated phosphorylation in the presence or absence of acutely added VX-445. Once the steady-state was reached, CFTR activity was determined by adding extracellular iodide and analysing the quenching of YFP-CFTR due to iodide/chloride exchange.

Our measurements revealed an enhanced VX-445-mediated potentiation in G551D in the R1030A-CFTR background, suggesting a synergy between the effects of VX-445 and of the R1030A mutation. The underlying mechanism may be similar to the one responsible for the synergistic co-potentiation between VX-445 and VX-770, as some evidence suggests that VX-770, like R1030A, enables non-canonical CFTR openings (Prins et al., 2020; J. Biol. Chem. 295:16529–16544). Further studies are planned to assess whether the R1030A mutation affects VX-770 activation of G551D-CFTR. These experiments could provide valuable insights into the mechanism of potentiation of VX-445.

P12

PF-429242 alleviates the p.phe508del-CFTR defects in cystic fibrosis cells

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Cystic fibrosis (CF) is the most common lethal autosomal recessive disease found in the European population. It is due to mutations in the *CF Transmembrane conductance Regulator (cftr)* gene coding for the CFTR protein, a chloride channel. The most common mutation in CF patients is p.phe508del-CFTR. The p.phe508del-CFTR protein is misfolded and retained in the ER. This protein is then rapidly degraded, which has an impact on its ability to be transported into the plasmic membrane. The main cause of morbidity and mortality in CF is the lung defect due to infection and inflammation due to a more viscous mucus production. Both are the main causes of the Unfolded Protein Response (UPR), a normal physiological process aimed to reduce the ER stress. It is regulated by three interconnected pathways: IRE1α, PERK and ATF6. Previous study showed that the activation of ATF6 downregulates CFTR's expression and we previously showed that siRNA against ATF6 allows CFTR channel-associated activity restoration. We found the same results using the PF-429242 (PF), an ATF6 pathway inhibitor. It is an anti-serine protease specifically inhibiting S1P which is essential for the ATF6's activation.

Our aim is to understand PF's molecular effects in a model closer to the pathology.

All results were obtained in CFBE410- cell line, which are human bronchial epithelial cells from a CF patient homozygote for F508-CFTR mutation. MTT tests were used to study PF's toxicity. Because ATF6's activation changes its localisation, we performed immunocytochemistry to localize ATF6 in cells after treatment. Western blots allowed us to study the cleavage of SREBP2 because this protein is cleaved and activate by S1P, as ATF6. We also study the expression of GRP78, a hallmark UPR protein, under PF and/or thapsigargin treatment by Western blot. We performed RT-qPCR to investigate 84 UPR specific gene expression. PCR array were used to study CFTR, IRE1, PERK, CHOP and XBP1 gene expression after PF treatment. CHOP and XBP1 are two important proteins in UPR. Immunoprecipitation allowed us to qualitatively see the total CFTR amount produced in cells. Patch-clamp experiments were performed to evaluate the rescue of p.phe508del-CFTR activity in our cell model, moreover we used Ussing chamber on human bronchi from CF patients.

We showed that PF is not toxic. ATF6 cellular localization is modified after treatment. It is absent from the nucleus showing its inactivity. Moreover, we saw by Western blot that PF inhibits the cleavage of SREBP2 indicating that PF is quite specific of S1P. CFTR mRNA and global protein amount were overexpressed after PF. We also highlighted 7 modulated genes (HSPA1B, CEBPB, VIMP, DNAJB9, INSIG1, HSPA5 and CALR) some of which are involved in CFTR synthesis, that could explain our results. Finally, we found that PF restores the Cl- channel function of p.phe508del-CFTR in cell line and in human bronchi.

In conclusion, we purpose PF as a new potential therapeutic molecule in order to increase CFTR synthesis and transportation into the plasmic membrane which result to an increase of chloride efflux in CF cells.

P13

T1a nanobody as a tool for studying the F508del mutation effect in NBD1

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Cystic fibrosis (CF) is caused by mutations in the cystic fibrosis transmembrane regulator (CFTR) a chloride channel regulating fluid transport in epithelial tissues. The most common mutation is the deletion of phenylalanine at the 508 position (F508del) in the nucleotide binding domain 1 (NBD1), which leads to channel degradation and lack of ion transport.

We developed a collection of nanobodies targeting human NBD1 (Sigoillot, M. et al, 2019) and identified nanobody T1a. This nanobody recognizes wt NBD1 and binds it with a high affinity (EC_{50} = 3 nM) but decreased affinity for F508del mutant or ΔRI (402-434 regulatory insertion removal). We obtained the crystal structure of ΔRI-NBD1:T1a complex which shows that the epitope includes F508. While it is known that F508del thermally destabilizes NBD1, we observe that T1a binding to NBD1 results in a lowering of the melting temperature of the complex. In addition, transfecting T1a in cells expressing wt CFTR leads to destabilization of the channel at the cell surface. We hypothesize that T1a binding to NBD1 mimics the effect of F508del on the domain, providing a unique tool to investigate the molecular basis of the CF-causing mutation.

In our research, we want to trace how F508del mutation leads to NBD1 destabilization using T1a nanobody. To catch changes, we use biochemical (ELISA), physical (single-molecule FRET (smFRET), thermal shift assay), and structural (X-ray crystallography, hydrogen-deuterium exchange mass spectrometry (HDX-MS) methods.

HDX-MS experiments reveal that T1a binding to NBD1 modifies the mobility of segments connecting the alpha-subdomain (containing F508) and β-subdomain while the same regions are known to be affected by F508del (Soya, N. et all, 2019). In addition, smFRET studies show that the conformational equilibrium of the RI domain is modulated by T1a binding similarly to F508del.

We identified key elements in the allosteric network of NBD1 that enable destabilization of the domain by the F508del mutation.

P14

Role of CFTR in airway epithelial regeneration and repair: studies from novel human basal cell lines

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Background: Remodelling and repair of the airway epithelium are key components of CF pathophysiology. Tissue repair, which starts by loss of cell polarity and involves several steps (migration, proliferation, adhesion, and re-differentiation) is critical to restore integrity and proper functioning of the normal airway epithelium after exacerbations caused by recurrent infections and chronic inflammation. Functional CFTR was shown to be essential for the rapid and correct regeneration of airway epithelium after injury [1]. Indeed, we recently demonstrated that wound closure after injury is delayed in CF primary human bronchial epithelial cells [2]. However, it is unclear whether this is directly driven by non-functional CFTR.

Objective: Exploring the role of CFTR in airway epithelial cell regeneration in human airway cells.

Methods: Human airway basal cells (BCi-NS1.1) have multi-potent capacity to differentiate into all airway epithelial cell types when cultured under air--liquid interface (ALI) on permeable supports for 30 days [3]. We assessed the expression of different cell-type markers by Western blot (WB) and immunofluorescence. Ussing chamber experiments were performed to analyse CFTR function. Wound-closure and regeneration capacity of fully-differentiated BCi-NS1.1 cells was monitored after injury by live-cell microscopy. Using CRISPR/Cas9 gene editing in BCi-NS1.1 cells we generated BCi-CF1.1 and BCi-CF1.2 cells expressing CFTR variants p.Phe508del (F508del) and p.Gly551Asp (G551D) in homozygosity, respectively, confirmed by Sanger sequencing.

Results: Results show that BCi-NS1.1 cells grown in ALI differentiate into the various airway cell types, including club (SCGB1A1); goblet (MUC5AC), and ciliated cells (DNAI1, FOXJ1, b-Tubulin IV). Importantly, BCi-NS1.1 cells also differentiate into tuft cells (POU2F3) and the rare high CFTR expressor ionocytes (FOXI1). Increased CFTR expression occurs during cell differentiation and functional analysis revealed a typical CFTR-mediated chloride secretion upon stimulation with IBMX/Forskolin, inhibited by CFTR-Inh172. Upon injury BCi-NS1.1 cells close the wound within 3 days. Importantly, levels of CFTR protein expression increase over time of wound closure and regeneration, suggesting a role of CFTR in these processes. To further study the role of CFTR, we successfully generated two novel isogenic basal cell lines, BCi-CF1.1 (p.Phe508del/p.Phe508del) and BCi-CF1.2 (p.Gly551Asp/p.Gly551Asp). Thus far, WB results show that BCi-CF1.1 cells also differentiate into the various airway cell types, including ionocytes, which remain unaffected in number vs non-CF. Ongoing studies are characterizing both BCi-CF1.1 and BCi-CF1.2 cells, including protein ZO-1 to assess tight junctions status, to explore the role of CFTR in cell differentiation/polarization and regeneration/repair.

Conclusion: The novel BCi-CF1.1 and BCi-CF1.2 cells constitute unique basal cell models allowing us to investigate the role of CFTR during cell-type differentiation, regeneration and possibly CF-related cancer.

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[1] Hajj *et al* (2007) *J Pathol* **211**:340350; Amaral *et al* (2020) *Int J Mol Sci* **21(9)**:3133.

[2] Quaresma *et al* (2020) *Cell Death Dis* **11(10)**:920.

[3] Walters *et al* (2013) *Respir Res* **14**:135; Simões *et al* (2019) *Life Sci Alliance* **2(6)**:e201900462.

P15

A PI3Kγ mimetic peptide promotes F508del-CFTR localization at plasma membrane through a PKD1-dependent mechanism

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Background: The most frequent mutation in cystic fibrosis (CF) patients (F508del-CFTR) results in a trafficking defect of the channel, that is retained in the endoplasmic reticulum (ER) and consequently degraded by activation of the ERAD pathway. Despite currently approved CFTR correctors have revolutionized the CF therapeutic landscape, these molecules displayed suboptimal efficacy in rescuing the trafficking of F508del-CFTR, underlying the need for additional approaches to enhance F508del-CFTR localization at the plasma membrane (PM).

Aims: Since cAMP is known to promote PM CFTR stabilization (*Lobo et al., J Cell Sci, 2016*), we hypothesized that the cAMP-elevating peptide we previously developed (PI3Kγ MP; Patent n° WO/2016/103176; *Ghigo et al., Sci Transl Med, 2022*) could be exploited to enhance F508del-CFTR PM density and increase the therapeutic effect of CFTR correctors.

Methods: Cell surface protein biotinylation and immunogold electron microscopy were used to evaluate CFTR PM density in HEK293T, 16HBE14o- and CFBE41o- cell lines upon PI3Kγ MP treatment. Phosphoproteomics was exploited to unravel potential targets activated by PI3Kγ MP.

Results: We confirmed our initial hypothesis, showing that PI3Kγ MP increased both wt- and F508del-CFTR PM density. However, we intriguingly observed that the effect on F508del-CFTR trafficking was independent on the ability of PI3Kγ MP to boost cAMP in the cell, suggesting an alternative underlying mechanism(s). An unbiased approach based on phosphoproteomics revealed protein kinase D1 (PKD1), a well-established orchestrator of protein trafficking, as being strongly activated by PI3Kγ MP. Interestingly, PI3Kγ MP failed to promote F508del-CFTR stabilization at the PM after PKD1 inhibition, thus revealing PKD1 as the key mediator of the effect of PI3Kγ MP. Finally, PI3Kγ MP synergized with approved cocktail of CFTR modulators Trikafta® (ETI) in increasing F508del-CFTR PM density, indicating that our peptide could be used to enhance ETI therapeutic effect.

Conclusion: This study identifies PI3Kγ and PKD1 as pivotal regulators of CFTR stability at the PM and suggests exploiting the ability of PI3Kγ MP to increase F508del-CFTR PM trafficking and ETI therapeutic effect.

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P16

Differential neutrophilic responses to *Pseudomonas aeruginosa* reveal novel protein-based treatments relevant to cystic fibrosis

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Cystic Fibrosis (CF) has devastating effects on approximately 70,000 patients worldwide by impeding the transport of fluids from the lungs, leading to chronic inflammation and persistent microbial infections. As a first line of defense against such infections, the host will produce and recruit immune cells (e.g., neutrophils); however, in CF patients, mutations within the cystic fibrosis transmembrane conductance receptor (CFTR) (e.g., ΔF508) render immune cells ineffective at clearing such infections, leading to life-threatening disease. Specifically, CF-associated neutrophils demonstrate impaired effector function compared to wild type neutrophils, causing altered antimicrobial protein and enzyme production. Such altered neutrophil activity in the lungs can permit the adaptation and survival of bacterial pathogens, including *Pseudomonas aeruginosa,* as well as the formation of biofilms, which are resistant to antimicrobial treatments. Presently, our understanding of the interactions between neutrophils and *P. aeruginosa* in a CF model are limited, reducing our ability to effectively treat the host and clear the infection.

In this study, we apply state-of-the-art mass spectrometry-based proteomics to define a baseline of core proteome signatures and unique protein profiles between wild-type (HL60-WT-derived) and CF-associated (HL60-ΔF508-derived) neutrophils under uninfected conditions. We identified 105 host proteins with a significant increase in abundance in the wild-type neutrophils compared to ΔF508 neutrophils, including neutrophil defensin 3 and gamma-enolase. Conversely, we observed 65 proteins with a significantly higher abundance in ΔF508 neutrophils compared to WT, such as galectin 1 and argininosuccinate synthase. Next, to evaluate how neutrophil protein production impacts clearance of *P. aeruginosa* biofilms, we performed quantitative proteomics profiling on WT and ΔF508 neutrophils following incubation with *P. aeruginosa* biofilms. Here, our experiments identified many neutrophil proteins with significantly altered abundance profiles across the conditions, including bactericidal permeability-increasing protein and cathepsin G, which were elevated in WT neutrophils during infection. Importantly, our dual perspective proteomics profiling (i.e., detecting both host and pathogen proteins in a single experiment) also revealed significant changes in the bacterial response profiles between the tested neutrophils, suggesting that the interactions between neutrophils and *P. aeruginosa* biofilms are also critical to driving infection. We support our observations with assessment of the WT vs. ΔF508 neutrophils in clearing *P. aeruginosa* biofilms using crystal violet assays and colony forming unit (CFU) counts to quantify biofilm disruption. Next, host proteins with known or predicted roles in bacterial clearance (e.g., enzymes, immune-associated) will be prioritized for *in vitro* purification and further characterization to identify candidates' leading to enhanced biofilm disruption. To further analyze protein candidates' influence on host-pathogen interactions scanning electron microscopy (SEM) and biofilm disruption assays will be performed to validate these findings.

Together, this work enhances our understanding of biological mechanisms underpinning the host's ability to clear bacterial infections. Further, these findings support the discovery of potential novel therapeutic strategies to clear *P. aeruginosa* biofilms and aim to improve the quality of life for patients and their families living with CF.

P17

Effect of the lipid environment on the F508del CFTR rescue and stability at the plasma membrane

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The CFTR channel exerts its activity at the plasma membrane (PM) level within specific microdomains whose lipid pattern results altered in bronchial epithelial cells derived from patients with cystic fibrosis (CF).

Interestingly, we found that in CF bronchial epithelial cells carrying F508del CFTR the partial restoration of the CFTR lipid environment by administration of the ganglioside GM1 improves the stability of the mutated channel rescued by the treatment with Orkambi[(1)].

The new triple combination of CFTR modulators, Kaftrio, shows a higher efficacy in the rescue of F508del CFTR even if the infection with *Pseudomonas aeruginosa (P.a.)* determines an important destabilizing effect of the rescued mutated channel[(2)].

By analysing the sphingolipid pattern of CF bronchial epithelial cells overexpressing F508del CFTR and treated with Kaftrio, we observed a partial increase in the content of certain gangliosides, such as GM1 and GD1a, that could be, in part, responsible for the higher efficacy of the triple combination. These changes appear to be mainly due to a promotion in the activity of the sialyl-transferase and to the inhibition of the sialidase, two key enzymes involved in the ganglioside metabolism. Conversely, the levels of cholesterol, another lipid normally enriched in lipid domains and promoting the function of CFTR [(3,4)], were found to be decreased in cells treated with Kaftrio.

Based on this evidence, we investigated whether restoring the lipid composition of the CFTR microdomain by administration of GM1 and cholesterol could ameliorate the effectiveness of Kaftrio on rescuing F508del CFTR, also in presence of *P.a.* infection. For this reason, we fed CF bronchial epithelial cells overexpressing F508del CFTR, treated with Kaftrio in presence or not of *P.a.*, either with different molecular species of GM1 or with human lipoproteins with the aim to deliver cholesterol to the cells.

As a result of these experiments, we observed that GM1 administration improves the effect of Kaftrio in term of increased levels of the rescued F508del CFTR at the PM level, and by using different molecular species of GM1, we demonstrated that those carrying shorter acyl chains result to be more effective. Moreover, the GM1 administration partially counteracts the CFTR instability induced by *P.a.* infection.

On the other hand, we found that the treatment of cells with lipoproteins, particularly LDL (low-density lipoprotein), determines a relevant increase in the CFTR levels.

Based on these data, the administration of GM1 and/or cholesterol, together with correctors and potentiators, could be considered as an innovative strategy to ameliorate the effectiveness of CFTR modulators and could also open a new scenario for the treatment of CF patients with orphan mutations affecting folding and stability of the channel.

1. Mancini G, Loberto N, Olioso D, *et al*. Int J Mol Sci (2020)

2. Stanton BA, Coutermarsh B, Barnaby R, *et al*. PLoS ONE (2015)

3. Abu-Arish A, Pandzic E, Goepp J, *et al.* Biophys J (2015)

4. Chin S, Ramjeesingh M, Hung M, *et al.* Cells (2019)

P18

Non-canonical inhibitors target CAL binding: new approaches to stabilize functional F508del-CFTR

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The era of highly effective modulatory therapies (HEMTs) requires a comprehensive reassessment of therapeutic strategies for people with CF. While HEMTs have provided substantial improvements in lung function and reductions in pulmonary exacerbations requiring hospitalization, responses vary widely among individuals, and there is clear room for continued improvement and personalized optimization of combination therapies. Such improvements might also address the persistence of chronic airway infections in many patients.

Our prior work has established that endogenous trafficking proteins can serve as therapeutic targets that complement current drug combinations, which consist of corrector and potentiator modulators that address the processing and gating defects of F508del-CFTR, respectively. While highly effective for many patients, approved therapies do not directly address the reduced plasma membrane stability of rescued F508del-CFTR. Thus, we aim to identify drugs (stabilizers) that increase the plasma membrane half-life of CFTR by targeting the CFTR-Associated Ligand (CAL) PDZ domain, an essential mediator of CFTR lysosomal degradation.

Protein-protein interactions are notoriously difficult drug targets. The surfaces are distributed and relatively featureless, forcing trade-offs between potency and specificity, and thus complicating efforts to develop inhibitors. Here, we report a combination of screening, structure-based design, computational modeling, and biochemical and cellular characterization to develop peptide and small-molecule inhibitors of the CAL PDZ domain, as a basis for further development. Our best CAL PDZ peptide inhibitors exhibit single-micromolar affinities for the target binding site. However, they also interact with off-target PDZ domains, some of which may have countervailing negative effects on CFTR stability. We have identified a highly specific variant, but is 10x less potent. To improve affinity without losing specificity, we have now modeled and synthesized non-natural amino acids to reach outside of the canonical peptide binding site, contacting PDZ loops that are more variable, and thus potentially more selective. We report here proof-of-principle using a novel adducted lysine side chain that enhances peptide affinity by forming a stereochemically specific interaction with a distal loop. Our second strategy has been to screen libraries of drug-like small molecules, using a combination of fluorescence-based high-throughput assays. Candidates were selected for further study based on follow-up dose-response studies and biochemical validation. We identified a family of covalent inhibitors that bind a cysteine residue outside the binding site and undergo further chemical modification. Detailed biochemical studies reveal that a specific protein state is inhibited, laying the groundwork for development of more precisely targeted reagents and follow-up functional and preclinical analyses to determine additive or synergistic efficacy as CFTR stabilizers.

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P20

Evaluating the role of mucins in the defective clearance of *Aspergillus fumigatus* conidia in the cystic fibrosis lung

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Aspergillus fumigatus is a frequent coloniser of the cystic fibrosis (CF) lung and is responsible for the development of several diseases including allergic bronchopulmonary aspergillosis (ABPA) and aspergillosis bronchitis (AB). Our understanding of the factors that drive *A. fumigatus* infections in CF is limited. However, we hypothesise that the increased incidence and pathogenicity of *A. fumigatus* in CF patients is in part due to the host's inability to clear fungal conidia as effectively as healthy individuals.

To test this, we first established a new *in vitro* model of isogenic CF and healthy control airway epithelial cell lines by using CRISPR/Cas9 mutagenesis to knock out *CFTR* in A549 cells. We confirmed KO of CFTR using western blot and characterisation of the model revealed several phenotypes including a reduced ability of the cells to transport chloride ions and an increased production of glycoproteins including MUC5AC. This led us to investigate whether the elevated production of glycoproteins impacted the ability of *A. fumigatus* conidia to adhere to the epithelial cells, avoiding clearance. We utilised a microscopy-based approach to quantify the amount of *A. fumigatus* conidia adhered to cell monolayers, following 2 h incubation. However, results from these experiments revealed no difference in the ability of the conidia to adhere to the cell monolayer, suggesting that in this capacity, increased glycoproteins did not impact clearance of spores.

Following this, we aimed to investigate whether CFTR KO had an impact on the ability of the cells to internalise *A. fumigatus* conidia. For this, we employed a single-cell approach based on Imaging Flow Cytometry (IFC) to measure internalisation of *A. fumigatus* conidia. Here, we demonstrated significant differences in the ability of CFTR KO cells to internalise *A. fumigatus* spores; only 6% of infecting spores were internalised by CFTR KO cells, compared to 13% for the wildtype. Our data therefore demonstrates that the internalisation process in CFTR KO cells is aberrant, suggesting a role in the promotion and establishment of *A. fumigatus* infection in the CF lung via the dysregulation of fungal clearance mechanisms. Nonetheless, further work is needed to determine whether these differences are linked to the over-production of glycoproteins by the cells.

P21

Structure of domains in polymerizing mucins and their importance for the formation of normal and CF mucus

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The major constituent in all mucus is one or several gel-forming mucins. In the intestinal tract, it is only the MUC2 mucin whereas the respiratory tract normally have two mucins, the MUC5B and the MUC5AC. All these mucins are built by central PTS sequences that after dense O-glycosylation become extended, stiff mucin domain rods interrupted by CysD domains. The CysD domains are around 100 amino acid with five disulfide-bonds that stabilize a stalk made up by six antiparallel β-strands. The top of this stalk carries two flexible loops that extend outside the surface formed by the glycans taking part in pH-dependent dimerization.

The N-terminal part of these mucins are made up 3.5 von Willebrand D (VWD) assemblies, each built by a VWD domain, a C8, and a TIL domain. The first two WVD assemblies are important during intracellular packing in the goblet cell mucin granule, but it is the dimerized third VWD3 assembly that is important for the assembled mucus. The MUC5B mucin is only forming the disulfide-bonded dimers and thus linear polymers. In the MUC5AC on the other hand, the VWD3 forms additional non-covalent interactions to generate net-like polymers, interactions that vary in strength depending on genetic SNP variability.

The C-terminal part of these mucins are made up by one VWD assembly, 3.5 VWC domains and finally a C-terminal cystine knot domain. The structure of the MUC2 C-terminus has been determined and show as the other mucin a tightly held together extended dimeric structure. All are dimers due to the cystine-knot domain, but the MUC2 is also stabilized by an extra disulfide bond before the C-terminal VWD.

Interactions between these different domains determines the packing of the mucins at low pH intracellular and the formation of the mature mucus after secretion and expansion as triggered by bicarbonate and increased pH.

P22

Understanding the roles of ionocytes in the airway epithelium using hiPSC-derived airway epithelial cells

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CFTR malfunction in Cystic Fibrosis (CF) leads to increased mucus viscosity which reduces clearance in the airway epithelium and predisposes to bacterial infection and biofilm formation. Pulmonary ionocytes are a rare population of ion exchanging cells that express high levels of CFTR. Because of this, they have been hypothesised to have a key role in the physiopathology of CF, although their exact function in the epithelium remains unknown.

The limited availability of human airway tissue and the scarcity of ionocytes in the epithelium have made it challenging to study ionocytes. For this reason, we decided to take advantage of human induced pluripotent stem cells (hiPSCs) to derive airway epithelium that can be used to study the airway epithelium and for disease modelling. Importantly, hiPSCs are easy to genetically modify, providing a platform to study genetic diseases and to perform loss of function assays. However, currently available protocols for airway epithelial cell (AEC) differentiation often fail to demonstrate the presence of rare cell types such as tuft cells and ionocytes. Thus, our group has developed a protocol to efficiently differentiate hiPSC into AEC cultures that contain ionocytes.

Our protocol mimics embryonic development of the airway in vitro by subjecting the cells to differentiation towards definitive endoderm, anterior foregut endoderm and lung progenitor state. At this point, lung progenitors are isolated using a sorting step and there grown as 3D organoids. These cells can be cryopreserved, which bypasses the need to re-differentiate hiPSCs for each experiment. Functional maturation of these cells in air-liquid interface leads to a polarised pseudostratified epithelium with basal cells, ciliated cells, secretory cells and ionocytes as shown by qPCR and/or immunocytochemistry analyses. Importantly, these hiPSC-AEC cultures show barrier properties and ciliary beating frequencies similar to those derived from primary bronchial epithelial cells.

To investigate the role of ionocytes, we generated hiPSC lines knock out for FOXI1, a transcription factor essential for ionocyte differentiation. FOXI1^{KO} hiPSCs were differentiated into AECs and, when compared to their isogenic wildtype control, we observed no differences in differentiation efficiency up to the lung progenitor state. Upon maturation, although the expression of key mature cell markers was not statistically different, flow cytometry assays revealed a lower number of FOXJ1-expressing ciliated cells in FOXI1^{KO} cultures. In terms of functionality, analysis of the ciliary beating frequency by a robust Fourier Transform method showed that FOXI1^{KO} cultures displayed slower-moving cilia. This analysis also confirmed a lower cilia coverage in FOXI1^{KO} cultures. Ongoing experiments exploring epithelial composition and cilia morphology, will help elucidate the exact mechanisms by which ionocytes play a role in the composition of the airway epithelium.

Taken together, these results demonstrate the interest of hiPSCs to study the function of human ionocytes in cilia motility in health and in disease thereby paving the way to more targeted treatments. Furthermore, the model has the potential to be useful for the study of other lung diseases and to bring us a step closer to regenerative therapy.

P23

Sputum viscoelasticity is driven by concentration in organic components

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Sputum from Cystic Fibrosis (CF) and Non-Cystic Fibrosis Bronchiectasis (NCFB) patients have high viscoelastic properties which complicates its clearance leading to airways obstruction and infection. Sputum viscoelasticity has been shown to correlate to solid fraction. However, it remains unclear whether this correlation is primarily driven by organic (mucins, DNA, cell debris, etc.) or inorganic (salts) content. Here we quantify separately these two influences in CF and NCFB sputum samples.

CF and NCFB adult sputum samples were collected from the “Centre de Ressource et de Compétence de Mucoviscidose" (CRCM) in the Grenoble Alpes University Hospital (CHU). The study was approved by the Comité de Protection des Personnes (case number 20.09.08.61213). Expectorations were induced by drainage with the help of a physiotherapist and freeze dried prior to characterisation. Sample’s solid concentration was measured by drying at 37°C during 24h and weighting it in a precision balance, and salt concentration was obtained by conductimetry. Furthermore, rheometry was measured for each sample at low and high deformation obtaining the viscoelastic modulus (G*) and the critical strain and stress (and), respectively. Finally, an NCFB expectoration was gradually diluted at isosalinity to assess if the variability between patients could be retrieved by simply tuning the sample’s hydration.

Total solid concentrations of CF (n=13) and NCFB (n=15) samples ranged between 8.42 and 104.29 mg/mL and 21.3 and 86.96 mg/mL respectively, and the salt amount was 5.62 ± 0.44 mg/mL in CF and 7.94 ± 1.73 mg/mL in NCFB expectorations.

We observed a linear correlation between G* and the organic solid concentration, and no significant dependence to salt concentration.

Remarkably, the correlation between G* and organic solid concentration was identical between CF and NCFB. Moreover, the hydrated sample also fell onto the same correlation, which suggests that the inter-sample’s variability in G* is merely caused by the proportion of water and organic solids, regardless of the detailed composition of the sample.

This study has therefore confirmed the influence of organic solid concentration on sputum’s viscoelasticity while showing no evidence of salt influence.

P24

Olive Leaf Extract (OLE) as novel antioxidant that ameliorates the inflammatory response in cystic fibrosis

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ßProlonged *P. aeruginosa* infections have been linked to chronic inflammation in Cystic Fibrosis (CF) lung, worsening the damage to lung tissue by neutrophil-derived reactive oxygen species (ROS) and proteases. Despite the approved highly-effective CFTR modulators restoring the CFTR defects and improving the CF lung disease, their anti-inflammatory and antioxidant potential has not yet been elucidated. Current anti-inflammatories are poorly effective and have dramatic side effects in CF patients. Hence, to identify innovative, effective, and safe drugs to improve redox imbalance and inflammation in CF patients is actually an unmet need.

Olive leaf extract (OLE) is known to contain large amounts of polyphenolic compounds with antioxidant, anti-inflammatory, and antimicrobial properties (Karygianni et al., 2014). Since OLE is used in traditional medicine for this triple activity, we were prompted to evaluate its effect on CF airway epithelial cells.

We first examined the OLE effect as an antioxidant in bronchial epithelial cells (CFBE) over-expressing F508del-CFTR by measuring the ROS content with a ROS-sensitive fluorescent probe (H2DCFDA). We observed a significant reduction of intracellular levels of ROS by OLE a dose-response manner in CFBE cells after infection (LPS-dependent) or inflammation (IL-1ß/TNFa) stimuli.

To investigate the anti-inflammatory activity of OLE in CF, we measured the mRNA expression levels of pro-inflammatory cytokines (IL-1ß, IL-6, IL-8 and TNFa) by RT-PCR. OLE significantly reduced the inflammatory response to LPS or IL-1b/TNFa stimulation in CFBE cells, mimicking the infection and inflammatory status of CF patients. We then evaluated the anti-inflammatory activity of OLE in patient-derived nasal epithelial cultures from three CF patients bearing *F508del* mutation. Interestingly, OLE treatment downregulated the pro-inflammatory cytokines mRNA levels, stimulated by LPS, in primary nasal epithelial cells isolated from CF patients.

Interestingly, we found that OLE restored elexacaftor, tezacaftor, and ivacaftor (ETI)-mediated F508del-CFTR function in CFBE cells and in primary nasal epithelial cells stimulated by LPS using the fluorometric imaging plate reader (FLIPR) assay.

To further provide insights on the mechanism of action of OLE, we investigated the mRNA expression of antioxidant (nuclear factor (erythroid-derived 2)-like 2: Nrf2; heme oxygenase-1 (HO-1)) and anti-inflammatory (NF-kB (p50 and p65 subunits)) markers by RT-PCR. Interestingly, OLE treatment upregulated Nrf2/HO-1 and downregulated NF-kB signaling in CFBE and primary nasal epithelial cells stimulated by LPS confirming its key role in antioxidant and anti-inflammatory pathways.

These finding provide strong evidence of OLE to prevent redox imbalance and inflammation that cause chronic lung damage by enhancing the antioxidant activity and attenuating inflammation in CF airway epithelial cells. Additionally, we propose that OLE might be used in combination with CFTR modulators therapy to improve their efficacy in CF patients.

P25

Therapeutic strategies favoring the restoration of airway epithelial integrity and function in cystic fibrosis

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Background: In CF airways, dysfunctional Cl- secretion and reduced mucociliary clearance favor bacterial infections contributing to progressive lung damage and secondary respiratory failure, which remain the first cause of morbidity and mortality. Several years ago, we thus decided to devote our efforts to identify novel therapeutic strategies to promote CF lung repair. We first discovered that the capability of CF airway epithelia to repair is less efficient, than in healthy controls, most likely due to the CFTR defect and bacterial infections, in particular with the main pathogens infecting CF lungs, i.e. *Pseudomonas aeruginosa* (PA) and *Staphylococcus aureus* (SA). Our work also unveiled that new treatments with potentiators/correctors aimed at rescuing CFTR, significantly enhance airway repair. Unfortunately, the CFTR rescue and repair improvement by CFTR modulators are dampened by infection.

Objective: Our main goal is to identify new therapeutic strategies, interfering with bacterial virulence, to improve the efficacy of CFTR-directed treatments and favor the repair of infected airways.

Methods: We are using patient-derived samples (including primary airway cells from CF patients harboring various types of mutations as well as living tissues from lung explants, available through our biobank), exposed to virulence factors secreted by PA and SA bacterial cultures. We are then investigating CFTR expression/function as well as epithelial injury and repair processes.

Results: We first dissected how CFTR function regulates airway epithelial repair processes. We also identified which virulence factors from PA (elastase) and SA (α-, β-hemolysin) are responsible for the deleterious impact of infection. Importantly, we identified new approaches, interfering with bacterial virulence, which efficiently prevented the negative effect of infection on airway epithelial repair. Moreover, we have data supporting that airway epithelial integrity could be further improved by combined treatments, including CFTR modulators (Orkambi, Symdeko, Trikafta) as well as activators of another class of ion channels involved in epithelial repair, i.e. K^+ channels.

Conclusions: Altogether, these precision strategies, combining treatments targeting both bacterial virulence and ion channels, may favor the functional integrity of damaged CF lung tissues from CF patients harboring various types of mutations.

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P26

Evaluation of phage interactions with host immune system in models of cystic fibrosis: one step toward phage therapy application

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The rise of multiple drug-resistant (MDR) bacteria, including *Pseudomonas aeruginosa* (*Pa*), complicates the treatment of patients with cystic fibrosis (CF). Recently, phage therapy has been explored as a potential alternative tool for patients with CF. This therapy has been used for decades in Eastern Europe to treat a variety of infections and thus it is considered generally safe. However, there are aspects that deserve to be further studied to make phage therapy a realistic therapeutic option. We have started investigating some of the still open questions about phage therapy, like what happens when phages get in touch with eukaryotic cells in wild-type (WT) and CF models, how phages elicit modulation of the host immune system and which phage component(s) activate anti-inflammatory cascade. To address these aims we are studying the fate of FITC-labelled phages after the injection in WT and CF zebrafish embryos or following incubation with WT or CF human cell lines specific for airway epithelium (Cu-Fi F508del) or innate immune system (THP-1 CFTR-blocked). Moreover, we are dissecting the molecular mechanisms through which phages modulate host immune system in WT and CF zebrafish embryos. We are taking advantage to transgenic lines that allow the direct visualization of innate immune cells (i.e. neutrophils, macrophages), that are conserved from zebrafish to human. We are also depleting each of the two populations to assess their contribution to the anti-inflammatory effects previously shown following phage administration in zebrafish embryos. Finally, we are isolating and purifying proteins from DEV, the most promising among the phages composing a four-phage cocktail (CK4), and test which exerts anti-inflammatory effects in the above-mentioned *in vivo* and *in vitro* models. The discovery of mechanisms involved in phage/host immune system interaction in normal and pathological conditions will be relevant for the CF community. Indeed, clinical trials to assess the safety and tolerability of an inhaled phage cocktail in patients with chronic *Pa* infections have been started. This work aims to clarify unsolved issues of phage therapy that cannot be addressed in patients, with the final goal to make it a reliable and safe therapeutic option.

P27

Effects of lumacaftor/ivacaftor (Orkambi) on the severity of acute pancreatitis

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Background and aims: Heavy alcohol intake is one of the most common causes of acute pancreatitis (AP). We have previously shown that ethanol (EtOH) decreases the expression and activity of the cystic fibrosis transmembrane conductance regulator (CFTR) which plays a key role in alcohol-induced AP development. Orkambi (Ivacaftor and Lumacaftor) is available to correct the impaired CFTR function and expression in cystic fibrosis patients.

Our **aim** in this study was to investigate whether Orkambi could be also beneficial in pancreatitis.

Materials and methods: Intact guinea pig pancreatic ducts (PDs) were treated with different concentrations of EtOH (30; 50; 100 mM) alone and in combination with Ivacaftor and/or Lumacaftor (1; 3; 5 and 10 μM) for 3, 7, 9 and 12 hours and CFTR expression and activity were evaluated by immunostaining and patch clamp, respectively. Experimental AP was induced in Orkambi-treated guinea pigs and standard laboratory and histological parameters were measured.

Results: Ivacaftor (10 μM) and Lumacaftor (10 μM) alone or in combination dose-dependently restored the localization and activity of the EtOH-damaged CFTR channel, during 12 hours of treatment. Oral administration of Orkambi decreased the severity of EtOH-induced pancreatitis.

Conclusion: We showed for the first time that Ivacaftor and Lumacaftor are able to restore the CFTR defect caused by alcohol and decreases the severity of pancreatitis. These results indicate that Orkambi may represent a novel therapeutic option in the treatment of AP.

P28

Upregulation of sodium-glucose cotransporter (SLC5A1) by IL-17A/TNF-α as an anti-bacterial mechanism

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Background: Treatment of bronchial epithelia with the combination of IL-17A plus TNF-α, two cytokines with a relevant role in cystic fibrosis (CF), induces a profound change of cell transcriptome with upregulation of genes coding for ion channels and transporters (Guidone et al., JCI Insight 2022). The overall result of IL-17A/TNF-α treatment is a marked hyperviscosity of airway surface fluid (ASF) as demonstrated by Fluorescence Recovery After Photobleaching (FRAP) experiments. Importantly, stimulation with a beta-adrenergic agonist switches ASF to a fluid state in non-CF but not in CF epithelia. The IL-17A/TNF-α treatment also upregulates genes coding for chemokines, defensins, and other potential anti-bacterial mechanisms. We were particularly interested in the upregulation of SLC5A1, which codes for an electrogenic sodium-dependent glucose transporter.

Aims: Our aim is to investigate the role of SLC5A1 upregulation by IL-17A/TNF-α as a potential anti-bacterial defense mechanism.

Results: We carried out short-circuit recordings on differentiated bronchial epithelia without glucose in the apical solution. Epithelia treated with IL-17A/TNF-α for 72 h responded to glucose addition (0.2 – 10 mM) in the apical compartment with a dose-dependent increase in transepithelial current. The maximal value of the glucose-dependent current was 16.5 μA/cm^2 with an EC_{50} of 1.88 mM. In contrast, epithelia kept under control conditions showed negligible response to glucose. The current activated by glucose was totally inhibited by mizagliflozin (10 μM), a selective inhibitor of SLC5A1. By immunofluorescence, we found an increased signal for SLC5A1 in the apical membrane of IL-17A/TNF-α-treated epithelia. We are carrying out experiments to measure the extent of glucose depletion in ASF by SLC5A1 and its ability to prevent bacterial colonization in CF and non-CF epithelia.

Conclusions: Our results show that SLC5A1 is markedly more active in IL-17A/TNF-α-treated epithelia with respect to control. Enhanced SLC5A1 activity may cause glucose deprivation in the ASF, a condition that creates an unfavorable environment for bacteria. Our results remark the importance of specific cytokines in boosting epithelial defense mechanisms. It will be essential to assess in future studies if and how such mechanisms are impaired in CF epithelia.

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P29

The triple combination therapy effect on bicarbonate transport in F508del respiratory epithelium – novel insight from pHStat studies

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Mutations in the *CFTR* gene lead to defect in Chloride (Cl^-) and Bicarbonate (HCO_3^-)transport, reduced ASL volume and acidicpH.Recent findings have shown that triple combination therapy induces the alkalinization of CF ASL upon inflammation, however the effect of CFTR correction on HCO_3^-transport remained unclear.

The aim of the study was to characterize the effect of triple combination (Elexacaftor, Tezacaftor, Ivacaftor ETI) therapy on HCO_3^-transport across F508del-CFTR respiratory epitheliumat baseline, in the context of inflammation and to relate this changes to ASL pH.

Respiratory Epithelial cells were obtained from healthy subjects (WT) and CF patients and grown on porous support. Prior experiments, fully differenciated cells were treated with DMSO (control),VX-445/661/770 with or withoutinflammatory cytokines (TNF-α and IL-17).HCO_3^-secretion rateswere monitored in Ussing chamber under open-circuit conditions by the pH-stat method.

There was minimal (0.01 μEq/cm^2h) or noHCO_3^-secretionacross DMSO treated CF cells. Pretreatment with VX-445/661/770 elevated HCO_3^-secretionrates to nearly 80% of that observed in non-CF cells. Forskolin enhanced HCO_3^-secretion rateswhich werecompletely inhibited by Inh-172. Inflammatory conditions alone and in the combination with VX-445/661/770 enhanced HCO_3^-secretion rates to levelsobserved in WT cells. Total inhibition of HCO_3^-secretionwas observed after addition of pendrin inhibitor (YS-01) and GlyH-101. The pH measurements showed that VX-445/661/770 treatmentonly slightly increase the ASL pH. Inflammatory conditions (with and without VX-445/661/770) resulted in ASL pH alkalinization to levels similar to that observed in WT cells.

VX-445/661/770 corrects CFTR-dependent HCO_3^-transport, however this correction is not sufficient to restore ASL pH to levels observed in WT cells.Inflammatory conditions enhanceHCO_3^-secretion and result in ASL alkalinization. Under inflammatory conditions, HCO_3^-secretion is mediated by CFTR, and pendrin.

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P30

Effect of an agro-based compound (A-bC) on remodelling and regeneration of airway epithelium in cystic fibrosis

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Background/Aims: In Cystic Fibrosis (CF), the airway epithelium is progressively damaged and exhibits large areas of remodelling such as basal or goblet cell hyperplasia, ciliated cell absence or epithelial desquamation, therefore leading to mucociliary clearance impairment and loss of respiratory function. Using *in vivo* and *in vitro* models, we have previously demonstrated that the regeneration of CF airway epithelium is altered and leads to the reconstitution of a remodelled epithelium, even in absence of infection and inflammation. Experimental protocols of chronic inflammation in non-CF airway epithelial cells and inhibition of endogenous CF cell inflammation allowed us to assign a role for transient CF cell inflammatory memory in basal cell hyperplasia and in the increased height of CF airway epithelium, to highlight the involvement of lung tissue inflammation in goblet cell hyperplasia, the ciliated cell differentiation default being independent of inflammation. The identification of pharmacological compounds favouring mucociliary clearance through an improvement of CF airway epithelial regeneration is therefore crucial for patients with CF. In this perspective, we examined the influence of an agro-based compound (A-bC) obtained by bacterial fermentation of wheat, on the regeneration of the human CF airway epithelium.

Methods: Airway epithelial cells obtained from nasal polyps from CF patients (F508del/F508del, n=3; F508del/W1282X, n=1) were cultured at the air-liquid interface for 35 days, in presence or not of A-bC and of Cytomix (TNFα, IL1-β and IFNγ combo) in the basolateral medium. Epithelial regeneration was then investigated by counting the number of basal, ciliated and goblet cells, epithelium height was measured in culture sections, while IL-8 secretion was assessed by ELISA.

Results: Measurements on haematoxylin/eosin-stained sections showed that A-bC decreased the CF airway epithelium height, even in presence of chronic inflammation. Quantification of immunofluorescence-detected CK13-positive and MUC-5AC-positive cells indicated a decrease in basal and goblet cell number, respectively, in A-bC-treated CF cultures, with no influence of Cytomix on this effect. The number of b-tubulin-positive ciliated cells was significantly increased in CF cultures exposed to A-bC, which also reduced IL-8 secretion by CF cells.

Conclusions: Our results show that A-bC avoids the inflammation-related CF airway epithelial remodelling, probably through the modulation of CF cell inflammatory phenotype. Moreover, A-bC improves the CF ciliated cell differentiation by a mechanism independent of its anti-inflammatory effect. These data allow us to propose A-bC as a pro-regenerative compound favouring key parameters for efficient mucociliary function in the CF airway epithelium.

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Phage therapy in cystic fibrosis: the roadmap to cross the finishing line

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The alarming diffusion of bacteria resistant to the antibiotics currently in use and the delay in the pipeline for the discovery of new efficient drugs is a serious problem for chronic infections such as those caused by *Pseudomonas aeruginosa* (*Pa*) in individuals affected by cystic fibrosis (CF). As an alternative to antibiotic treatments, the use of bacteriophages (phages) to fight bacterial infections has gained increasing interest in the last few years. Phages are viruses that specifically infect and multiply within the bacteria, without infecting eukaryotic cells.

As a proof-of-principle, the first step for a standardized pipeline for the therapeutic use of phages, is the development of phage collections that must be thoroughly characterized, with full genome sequencing to ensure the absence of the potentially harmful gene for the host. We have performed this step by isolating and characterizing four phages (two *Myoviridae* and two *Schitoviridae*) able to infect *Pa*. Then, it is necessary to assess the bacterial susceptibility to phages by plaque assay and growth kinetics, to evaluate the efficacy of the combination of phages and antibiotics, and to analyze phage interaction with the host immune system.

Firstly, we addressed the ability of our four phage cocktail in killing laboratory and clinical *Pa* strains with analyses on liquid culture and on biofilm. We further verified phage efficacy in mouse, *Galleria mellonella,* and zebrafish *in vivo* models. To get closer to the translation into clinics in a CF context, we applied phage therapy to a *CFTR*-loss-of-function (CF-*lof*), zebrafish, a quick and cheap model that recapitulates several phenotypical aspects of the CF human condition, such as the higher susceptibility to bacterial infection, the delay in the immune response and the constitutive inflammation. In all the tested models, our phage cocktail elicited a strong effect in reducing bacterial load and improving the survival of the infected animals. We also considered the efficacy of our phage cocktail in combination with antibiotics and in case of infection with *Pa* strains resistant to one or more of the four phages.

Finally, we addressed the interaction of our phages with the host immune system, using human bronchial epithelial cells F508del *CFTR* and the aforementioned CF-*lof* zebrafish model. We found that phages did not elicit an inflammatory response but, on the contrary, mitigate the hyper-inflammation presented by *CFTR* human cells and zebrafish embryos. We also investigated the mechanism through which phages act as anti-inflammatory agents.

The discovery of mechanisms involved in phage infection and host immune system interaction in normal and pathological conditions will be relevant for the CF community. Indeed, clinical trials to assess the safety and tolerability of an inhaled phage cocktail in patients with chronic *Pa* infections have been started. Our research aimed at clarifying some of the still open questions of phage therapy that cannot be addressed in patients, with the final goal to make it a reliable and safe therapeutic option.

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Inflammatory responses is variable across different clinical isolated exoproducts of *Pseudomonas aeruginosa* and is reduced by elexacaftor/tezacaftor/ivacaftor pre-treatment in primary nasal epithelial cells

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Chronic infection and inflammation are primary causes of declining lung function in Cystic Fibrosis (CF) patients. TRIKAFTA® (Elexacaftor-Tezacaftor-Ivacaftor) is an approved combination therapy for CF patients bearing the most common mutation, *F508del*, in the cystic fibrosis conductance regulator (CFTR) protein. However, clinical studies on TRIKAFTA® demonstrated that the response to treatments is variable among patients (Keating D., 2018). This high patient-to-patient variation in TRIKAFTA® response may feasibly be attributed in part to differences in types of microbial infections across patients and even within a single patient over time. It has previously been demonstrated that the modulator-mediated rescue of F508del-CFTR is reduced by *P. aeruginosa* bacteria in infected human bronchial epithelial cells (Stanton, B.A., 2015). Our aims were, therefore, to evaluate the effect of *P. aeruginosa* on inflammatory response and on F508del-CFTR rescue by TRIKAFTA® in airway epithelial cells.

We first tested the effect of clinical isolates exoproduct of *P. aeruginosa* obtained from 10 CF patients on inflammatory response in F508del-CFTR overexpressing CFBE cells. Interestingly, we found that IL-8 mRNA expression levels in F508del-CFTR CFBE cells, were clinical isolated exoproducts of *P. aeruginosa* dependent. Moreover, 6 of 10 clinical isolated exoproducts from *P. aeruginosa* decreased TRIKAFTA-mediated F508del-CFTR function in strain-specific manner by 20 to 51% using the fluorometric imaging plate reader (FLIPR) assay. We then studied nasal epithelial cultures from 10 CF patients who were F508del-CFTR heterozygous and treated with the clinical exoproducts of *P. aeruginosa* from the corresponding patient. Interestingly, we found a higher variability for IL-8 mRNA level and released protein level across different clinical exoproducts in primary nasal epithelial (HNE) cells. Moreover, TRIKAFTA® pre-treatment decreased the IL-8 mRNA levels in HNE cultures treated with clinical exoproducts of *P. aeruginosa*. Lastly, we found that treatment of HNE cells with the clinical exoproducts isolated from the corresponding patient, reduced the TRIKAFTA®-dependent F508del-CFTR function in 2 of 3 CF patients.

These finding support the concept that in vitro screening of patient-specific responses to CFTR modulators under infection conditions could prove valuable for personalizing combination therapy approaches to improve the efficacy of CFTR modulators in CF patients.

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Downregulation of MMP9 expression in leukocytes and plasma: potential biomarker predicting the efficacy of therapies in cystic fibrosis

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In recent years, the awareness of the significance of inflammation in cystic fibrosis (CF) and the finding that CFTR is also expressed in non-epithelial cells have raised interest in the role of immune cells in CF. Moreover, this cell type, being also easily and quickly isolable from patients, could be a useful cellular model to monitor the efficacy of CF therapies during clinical trials. Indeed, it is important to consider that *in vitro* modulators efficacy does not always reflect their *in vivo* efficacy. Therefore, it became necessary to find biomarker related to the individual patient's response.

In this context, we previously identified changes in specific proteomic profiles related to CFTR activity in CF leukocytes after *ex vivo* treatment with Ivacaftor. The results obtained showed the downregulation of proteins belonging to the leukocyte transendothelial migration, particularly the matrix metalloproteinase 9 (MMP9). Since the high expression of this protease potentially contributes to parenchyma lung destruction and dysfunction in CF, the downregulation of MMP9 could represent one of the possible positive effects of Ivacaftor in decreasing the disease progression.

In order to confirm these data also *in vivo*, we used leukocytes isolated from CF patients before and following Ivacaftor or Trikafta therapy, according to the genotype of the patient, and we measured both MMP9 levels by Immunoblotting and the CFTR activity by GST-HS-YFP assay. These data showed that the therapies promoted a decrease in leukocytes MMP9 levels together with a recovery of CFTR activity. Moreover, both parameters correlated with an improvement of clinical parameters.

To investigate if similar correlations could be directly detectable in blood of patients we assayed, on some plasma samples of CF patients treated with Trikafta, MMP9 activity by Zimography. The results obtained revealed that the MMP9 levels measured in plasma reflected the same trend observed in leukocytes. This result suggests that the downregulation of MMP9 expression could be a useful biomarker to monitor and predict the effect of therapies in CF patients.

Although additional studies are needed to confirm these data in a higher number of patients, downregulation of MMP9 expression could be considered a promising biomarker suitable for application in a clinical laboratory setting, in order to accelerate a much-needed personalized medicine approach in CF.

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Elexacaftor/Tezacaftor/Ivacaftor causes sustained reduction in systemic inflammatory markers across the first year of treatment

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Background: Uncontrolled and pro-longed inflammation is a major cause of lung damage in people with CF (pwCF). Clinical trials have demonstrated that the highly effective CFTR modulator combination Elexacaftor-Tezacaftor-Ivacaftor (Elex/Tez/Iva) improves lung function and quality of life, but the impact of this treatment on inflammatory pathways is unknown. We conducted a prospective observational study in pwCF. Patient serum was collected during the initial year of treatment and analysed for inflammatory biomarkers of CF disease.

Aim: To determine the impact of Elexacaftor-Tezacaftor-Ivacaftor triple combination CFTR modulator therapy on systemic inflammation in people with CF.

Methods: Eligible adults with CF were selected by genotype and recruited when commencing Elex/Tez/Iva therapy. Participants returned for review at 1 month, 3 months and 1 year after commencing therapy and research blood samples were collected at these time points. The inflammatory biomarkers calprotectin and C-reactive protein (CRP) as well as cytokines IL-6, IL-8 and IL-10 and MMP-10 were measured in patient serum by ELISA. Proteomic analysis of 10 modulator naïve people with CF were also analysed for inflammation and organ damage proteins at baseline and 1 year.

Results: There was a four-fold reduction in C-reactive protein in the initial 3 months after commencing treatment (n=73, p=0.0021). IL-6 was significantly reduced (n=49, p=0.0023) and correlated with CRP reduction, however, there was no significant change seen in circulating levels of IL-8 or IL-10. Both IL-6 and CRP remain significantly reduced at 1 year. MMP-10 was significantly reduced after 3 months treatment (n=48, $p<0.0001$) and remained significantly reduced at 1 year ($p < 0.0009$). Calprotectin, a biomarker of exacerbation risk, was significantly reduced (n=38, p=0.0028) after 3 months of Elex/Tez/Iva in patients who had not been on a previous modulator. pwCF who were previously treated with Tez/Iva and switched to Elex/Tez/Iva at the start of the study had lower levels of calprotectin at baseline. Calprotectin remained significantly reduced at 1 year Elex/Tez/Iva treatment (n=40 $p < 0.0009$). Proteomic analysis at 1 year showed a significant reduction in IL-6, IL-20 and MMP-10 (n=10) in those who were modulator naïve.

Conclusion: Significant improvements are seen in markers of systemic inflammation following Elex/Tez/Iva treatment. These appear to be sustained over the first year of therapy. Resolution of inflammation in CF may therefore contribute to the clinical improvements seen in pwCF commencing Elex/Tez/Iva.

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Investigation of the CFTR-TGFβ1 interaction in inflammatory and fibrotic processes in healthy and CFTR-mutated human bronchial epithelial cells

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Objective: Lungs of people with cystic fibrosis (pwCF) are characterized by chronic inflammation and progressive matrix remodeling. These processes are affected by genetic modifiers, such as Transforming Growth Factor β_1 ($TGF\beta_1$), which plays an important role in inflammation and fibrosis and is enhanced by an imbalance of proteases, e.g. neutrophile elastase (NE) and its inhibitor elafin.

Aims: Since our group has shown that impaired lung function in pwCF is linked to elevated $TGF\beta_1$ concentrations in sputum, we investigated the impact of (1) the CFTR-mutation itself on inflammatory and fibrotic processes, (2) the interaction of $TGF\beta_1$ and CFTR, and (3) the hypothesis if blockade of $TGF\beta_1$-abrogates the effect of CFTR mutations in human bronchial epithelial cells (HBE).

Methods: CFTR-diseased (F508del homozygous; CF-DHBE) and wildtype HBE cells (NHBE) were stimulated with $TGF\beta_1$ or vehicle. Gene expression and activation of Stat3 and NFκB-pathway, as well as inhibitors of proteases were analyzed by qRT-PCR or immunoblot.

Results:

(1) Gene expression of mediators of the NFκB-pathway (IKKα, p50, p65) and of Interleukin-1β (IL-1β) as a NFκB-activator were reduced in CF-DHBE when compared to NHBE. NLR family pyridine domain containing 3 (NLRP3) mRNA[Sv1] as an index of inflammasome-mediated inflammation and Stat3 pathway were increased and activated in CF-DHBE, respectively. Expression of elafin was lower in CF-DHBE than in NHBE.

(2) TGFβ1 had similar effects on NHBE as the described CFTR-mutation itself. Interestingly, $TGF\beta_1$-mediated blockade of Stat3 signaling was mitigated in CF-DHBE. Moreover, $TGF\beta_1$ had an additive effect on increased expression of NLRP3, connective tissue growth factor (CTGF)[Sv2] and IL-1β and on decreased expression of elafin in CF-DHBE.

(3) Blockade of $TGF\beta_1$ using Pirfenidone or a TGFβ-receptor 1 inhibitor partially reversed these findings in CF-DHBE.

Discussion: Our study does not only show CFTR-mutation-mediated effects on inflammatory pathways, but also a novel CFTR-$TGF\beta_1$ interaction modulating inflammatory and fibrotic response, potentially *via* inhibition of elafin in HBE. Restoring elafin levels as a possible therapeutic option to reduce pulmonary remodelling in CF is worth further investigation.

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Pulmonary ketogenesis promotes tolerance to *P. aeruginosa* infection in the CF lung

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Introduction: *P. aeruginosa* is a major opportunist that causes chronic respiratory infection in patients with CF. *P. aeruginosa* readily adapts to the CF lung to coexist with its host, establishing a favorable airway microenvironment that tolerates the pathogen burden. However, exactly how *P. aeruginosa* promotes this milieu remains poorly understood. Ketone bodies are typically liver metabolites that promote tissue homeostasis not only by fueling cellular bioenergetics, but also by desensitizing phagocytes to infection. Here, we established that *P. aeruginosa* exploits host ketogenesis to generate an airway milieu that tolerates infection in the CF lung.

Methods: In a murine model of pneumonia, we studied tolerance to a collection of 17 CF host-adapted *P. aeruginosa* isolates and *P. aeruginosa* PAO1 as control. Airway and systemic ketones were quantified by metabolomics. The ketogenic program of each lung cell during *P. aeruginosa* pneumonia was analyzed by single-cell RNA-Seq. Airway accumulation of cytokines and effector cells were examined by ELISA and multi-color flow cytometry, respectively. To establish a ketogenic environment in the lung, we fed animals a fatty acid-rich, carbohydrate low ketogenic diet in comparison to a fatty acid-low, carbohydrate rich control diet.

Results: Both the CF host-adapted *P. aeruginosa* isolates and PAO1 triggered airway accumulation of ketone bodies during pneumonia. The ketones induced by the CF host-adapted *P. aeruginosa* isolates were mainly derived from lipofibroblasts, whereas those associated with acute PAO1 infection were produced by the liver. We confirmed the presence of ketone bodies in airway fluids from CF subjects. We recapitulated this ketone-rich environment by feeding mice a ketogenic diet, and found that the CF host-adapted *P. aeruginosa* isolates exploited this milieu to suppress the inflammatory response to infection. Interestingly, we observed that exposure of PAO1 to ketone bodies limited its ability to expose LPS on the surface, which not only reproduced the less inflammatory phenotype found in the CF isolates, but also contributed to the establishment of tolerance to infection.

Conclusions: Our findings demonstrated that CF host-adapted *P. aeruginosa* isolates co-opted metabolic routes associated with preservation of airway bioenergetic function to both adapt to the lung and trigger tolerance to disease, such as ketone bodies.

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Fertility deficiency in female cystic fibrosis patients: determining the role of the uterus' endometrium using cutting-edge organoid models

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Introduction/objectives: Female CF patients suffer from sub- to infertility, often facing problems to become pregnant. Underlying reasons remain understudied. In particular, it is largely unknown whether dysfunction of the endometrium is involved, the womb's inner lining and key tissue for embryo implantation and development. Although pregnancy rates have increased since the new-era CFTR modulator therapies, their direct impact on the female reproductive tract, and particularly the endometrium, is unknown. These gaps in the CF domain are mainly due to lack of appropriate study models. Therefore, we develop endometrium-derived, tissue-mimicking organoid models which are applied to decode the role of the endometrium in CF fertility deficiency and the impact of clinical CFTR modulators on endometrial (dys-)function.

Methods: We establish endometrial organoids (EMO) from both *Cftr*^{tm1Eur} mouse and human CF patients. The organoids are molecularly and functionally compared to healthy EMO to uncover aberrations that underlie CF fertility deficiency. FIS, Ussing chamber and HS-YFP quenching assays are used to measure CFTR function in the organoids and their response to CFTR modulators. To assess differences in endometrial responsiveness to estrogen (E2) and progesterone (P4), which *in vivo* regulate the estrous or menstrual cycle, EMO cultures are exposed to defined E2/P4 regimens. Expression of CFTR, endometrial functionality/fertility markers as well as inflammatory genes is investigated by RT-qPCR and immunofluorescence staining. Multiple CF tissues are indeed characterized by a (hyper-)inflammatory phenotype which in the endometrium would be an important contributor to dysfunction.

Results: First, we established EMO from *Cftr*^{tm1Eur} mice, which show a smaller lumen than wildtype (WT) EMO and do not swell in the FIS assay, both validating the CFTR defect. When treated with CFTR modulators, organoid swelling is rescued. Currently, we are comparing expression of endometrial functionality/fertility markers and inflammatory genes in CF *versus* WT EMO, as well as their responsiveness to E2 and P4 (mimicking the different estrous cycle phases).

Second, we develop(-ed) organoids from CF patient endometrium. Before, we have shown that EMO from healthy endometrium can reliably reproduce all menstrual cycle phases under defined E2/P4 exposure. These organoids show phase-dependent *CFTR* expression similar to *in vivo*. Moreover, functionality of the expressed CFTR (in healthy EMO) was confirmed by HS-YFP quenching and Ussing chamber assays. Currently, we are deciphering whether and how menstrual cycle phases, including the embryo receptivity stage, are different between CF and healthy EMO. First experiments show dissimilarities in the proliferative and secretory phase, such as increased apoptosis and decreased fertility marker expression, respectively, thus providing a first indication of aberrant hormonal response in human CF endometrium. Now, the impact of CFTR modulators is being assessed.

Discussion: We establish(-ed) organoid models from CF endometrium as novel and powerful tools to gain insight into the endometrial factor in CF fertility deficiency. Importantly, the organoids are highly apt to explore the impact of CFTR modulators on the endometrium, at present unknown. Moreover, our study has the potential to reveal paths toward restoring reproductive fitness in CF patients, which can be tested using the organoids as (drug) screening platform.

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Proteomic profiling of sweat in patients with cystic fibrosis, effect of CFTR modulators

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Although sweat is a reliable, non-invasive, and easy to collect biofluid, sweat proteome profiles as a source of biomarkers has been studied in relatively few diseases. In a recent study, we compared sweat proteome profiles between cystic fibrosis (CF) patients and healthy controls and revealed a unique subset of differentially expressed proteins. These results provided new insights into epidermal homeostasis and a potential implication of the defective CFTR in a rare dermatological condition, aquagenic palmoplantar keratoderma, frequently observed in cystic fibrosis. CF sweat proteome highlighted an unbalanced proteolytic and proinflammatory activity of sweat in CF. These results also suggested a defect in pathways involved in skin barrier integrity in CF patients. This was the first in-depth characterization of sweat proteome profiles and suggested that sweat proteome profiles could serve as convenient tools in cystic fibrosis for diagnosis or personalized therapeutic interventions.

The present study compared sweat proteome profiles in CF patients after a 2-month treatment with ivacaftor-lumacaftor. 20 µL of sweat was collected after pilocarpine iontophoresis during a sweat test and liquid chromatography tandem mass spectrometry proteomic analysis was performed. Although a significant decrease of sweat chloride was observed after treatment**,** the subset of differentially expressed proteins previously identified was unchanged by the treatment. Although ivacaftor-lumacaftor treatment did not change the proteome profile, sweat remains an easy to access and informative biofluid. Further studies with the triple combination ivacaftor/tezacaftor/elexacaftor could reveal a greater variation in proteome profiles or help differentiate subsets of patients.

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Hope for European CF patients with rare mutations through organoid-based personalized medicine

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Cystic fibrosis (CF) is the most common, life-limiting autosomal recessive disease in the Caucasian population, affecting approximately 80,000 people worldwide, of which 50,000 people live in Europe. The vast range of genetic diversity contributes to a complex array of clinical progression and comorbidity. The CF community is celebrating the clinical impact of the approved Kaftrio, ELX/TEZ/IVA (ETI) triple-combination therapy for individuals with specific CFTR mutations. ETI has been the most efficacious combination so far, prompting remarkable improvements in many biomarkers.

Nearly 30 % of cystic fibrosis patients in southern European countries are still waiting for effective pharmacological therapy. Among them, many cannot wait for new drugs to be developed because of their clinical need. The next assignment is objectively to develop a path to access and reimburse ETI for patient groups who show a positive response to therapy in patient-derived cells. This is being addressed by a European-funded project, Human Individualized Treatment for CF (HIT-CF), using organoid technology to match patients to drugs. Such functional validations through in vitro genotype-phenotype studies are particularly useful in evaluating therapeutic agents in cancer and cystic fibrosis, where drugs are designed to target certain mutations and are not assumed to be widely beneficial. As a proof of concept, we evaluate individualized responses to ETI and an experimental therapeutic intervention, PTI triple-combo (PTI428/PTI801/PTI808) by Fair Therapeutics Inc., in intestinal organoids of 30 CF patients with any modulators approved for their rare mutations. By FIS (Forskolin-induced Swelling) assay, we could confirm the activity of ETI in 27 patients out of 30. The organoid response was higher than the average response of the combination ETI in F508del/G542X organoids for subjects with G85E/G542X, Q220X/A1006E, L1077P/N1303K genotypes and in one patient homozygous for the rare complex allele [R117L; L997F]. We identified candidate mutations for ETI, such as N1303K, 711+3A->G, R1066C, and A1006E. Based on in vitro results, twelve subjects were recently enrolled in the VX21-445-124 trial and will start the modulator treatment ETI in the upcoming months. Lower responses to those observed with ETI were measured in the presence of PTI triple-combo, except for subjects with G85E/T338I, R347P/P5L genotypes. We are currently using this approach to validate tissue samples as predictive of how patients react to drugs in vivo, developing a personalized path where governments and/or private insurers support expanding access to efficacious medications.

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Investigation of small nitrogen heterocycles as class 3 correctors in the rescue of mutant CFTR in cystic fibrosis

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Background: Although significative advances have been obtained in the pharmacological treatment of CF by combinations of correctors with complementary mechanism, new modulators are still needed to maximize the rescue F508del and other CFTR mutants with trafficking defects. In the course of our previous studies, we have identified a class of new small molecules (PP compounds), as correctors of F508del-CFTR on native epithelial cells of CF patients, particularly in combination with class 1 correctors (VX-809).

Aims: Since the initial discovery of the first active PP compound, we have synthetized more than 200 derivatives that, after functional tests, have provided an insight of the structure-activity relationship. Along with chemical manipulation of PP scaffold, a scaffold hopping (SH) approach generated new analogues based on a free rotable structure. Our objective is a multiparametric optimization of PP and SH compounds, to generate candidates with improved features, in terms of corrector potency/efficacy, and of drug-likeness.

Methods: The new synthetized compounds were tested by HS-YFP assay on cells with stable expression of mutant CFTR. The most active compounds were then validated: i) in short-circuit recordings on primary airway epithelial cells (bronchial and/or nasal); ii) in biochemical and microscopy assays to evaluate CFTR maturation/trafficking.

Results: Following iterative cycles of chemical synthesis, the optimization of ADME profile was achieved by: 1) introduction of hydrogen bond donors and acceptors in different regions of the scaffold and 2) a scaffold hopping strategy. In several cases, a reduction of the calculated lipophilic character (LogP) was accomplished maintaining the synergistic effect. Furthermore, the evaluation of corrector activity indicated that PP compounds act as class 3 correctors, since they induce synergistic effect when combined with class 1 (VX-809) and 2 (3151) correctors but not with class 3 (4172).

Conclusions: We have identified novel CFTR correctors, with promising features for the development of combinatorial treatments for CF. The optimization process is ongoing, in order to obtain the best trade-off between potency/efficacy and "drug-likeness" to develop an optimized lead compound that could be considered for preclinical and clinical development.

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P46

A novel CFTR corrector displaying a unique mode of action

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P47

Improving N1303K-CFTR modulator rescue by screening for compounds that enhance plasma membrane expression

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Introduction: Cystic fibrosis (CF) is a severe monogenic disorder caused by mutations in the CFTR (CF transmembrane conductance regulator) gene. CFTR modulators (correctors/potentiators) have been developed to overcome the protein dysfunction. N1303K is the fourth most common CFTR mutation (1.6% of CF alleles) and the second most common mutation without approved causal therapy. The triple combination teza-/elexa-/ivacaftor (Trikafta(tm)/Kaftrio(tm)) is currently not approved for N1303K due to the limited rescue observed in vitro, although a clinical trial is currently ongoing (NCT03506061). N1303K has been shown to reduce CFTR plasma membrane (PM) expression and impair its gating. While existing potentiators are able to enhance N1303K function, the major bottle neck is absence of potent correctors that can increase the amount of PM localized CFTR amendable to potentiation.

Aims: We hypothesized that repurposing of F508del correctors might not necessarily lead to the most optimal compounds for N1303K, given that both mutations lead to very distinct processing defects. To this end, we set up a medium throughput trafficking assay in A549 cells to identify novel small molecules able to enhance N1303K PM expression. In order to assure functionality was retained in a primary cell model, confirmed hits were validated in patient-derived rectal organoids harboring the N1303K mutation.

Methods: We developed a high content imaging-based screening assay to identify compounds able to increase N1303K-CFTR PM density in A549 cells, using an extracellular triple hemagglutinin (3HA) tag for specific PM labeling. A repurposing library of 2960 compounds was screened at 20 µM and compounds that improved PM density above lumacaftor were selected. After hit confirmation, their efficacy and toxicity were evaluated. Compounds were next tested in primary rectal organoids homozygous for the N1303K mutation. Here, CFTR functional rescue was measured by forskolin induced swelling (FIS) for 1 hour in the background of iva-, elexacaftor (co-)potentiation or the triple combination teza-/elexa-/ivacaftor (Trikafta(tm)/Kaftrio(tm)).

Results: We set up a robust ($Z' = 0.64 \pm 0.10$) high content analysis screen in order to identify hits capable of improving N1303K-CFTR PM expression. 10 compounds were selected based on their ability to improve N1303K PM density between 1.6 and 5.3□fold, similar to or above the level of correction by lumacaftor. Most compounds had an EC_{50} in the low micromolar range and showed no toxicity at these concentrations. One compound was additive to teza-/elexa-/ivacaftor in N1303K homozygous organoids, an effect which was at least partially acute. Investigation of the mechanism(s) of this compound is currently ongoing.

Conclusions: We aimed to identify compounds improving N1303K trafficking, in order to increase the pool of PM localized CFTR, amendable to potentiation. One compound, identified in a PM density screen in A549 cells, improved N1303K functional rescue on top of the Trikafta(tm)/Kaftrio(tm) combination teza-/elexa-/ivacaftor in primary rectal organoids. This work expands the knowledge on treatment options for one of the more common *CFTR* mutations, N1303K, which causes a severe form of CF, and to date has no approved CFTR modulator drugs available to tackle the cause of the disease.

P48

Theratyping of the rare CFTR genotype A559T/A559T in rectal organoids and nasal cells reveals a relevant response to the combination of elexacaftor and tezacaftor

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Background: Impairment of expression, trafficking and activity of the cystic fibrosis transmembrane conductance regulator (CFTR) channel by loss-of-function CFTR variants causes cystic fibrosis (CF). Small-molecules compounds correcting the underlying defect(s) of mutant CFTR channel became a useful/successful therapeutic treatment of CF. We aim at identifying CF subjects with rare/uncharacterized CFTR variants that may benefit from CFTR-modulators therapies. In this study we used rectal organoids and nasal brushing epithelial cells (NBEC) from a black CF patient (African origin, Ghana) homozygous for A559T (c.1675G>A) CFTR variant to evaluate the responsiveness to lumacaftor (LUMA), tezacaftor (TEZA) and tezacaftor+elexacaftor(ELE). A559T is a very rare variant, found in North American black CF patients (https://cftr2.org). This missense variant results in full-length CFTR expressed in reduced quantity on the cell-surface, due to a severe maturation defect (class II) (Gregory RJ et al., 1991). FRT cells expressing A559T-CFTR were reported as not responsive to IVA treatment (Van Goor et al.,2014). At present, there is no treatment approved for this variant. This patient, 31 years old, was enrolled in HIT-CF study(www.hitcf.org); due to his current severe clinical conditions he is not candidate for phase 2 of this study that proposes treatment of patients with experimental CFTR-modulators based on the response in their rectal organoids.

Methods: CFTR function was measured by short-circuit current (Isc) after treatment with LUMA/TEZA/ELE (Vertex Pharmaceuticals) in 2D rectal organoids (colonoids) and NBEC. The effectiveness of CFTR modulator is also being evaluated by forskolin-induced swelling assay and expression of mature CFTR (Western blotting) using 3D rectal organoids.

Results: In vitro drug response by short-circuit current measurements indicate that A559T-CFTR presents a minimal function in colonoids (ΔI_{sc}0.9 ± 0.3 μA/cm^2) and NBEC. The acute addition of IVA following CFTR activation by forskolin had no significant increment of baseline level of chloride transport in colonoids (ΔI_{sc}1.6 ± 0.2 μA/cm^2) and NBEC as reported by others. Moreover, there was no significant augment of A559T-CFTR channel activity in the presence of correctors LUMA (ΔI_{sc}1.4 ± 0.3 μA/cm^2) and TEZA (ΔI_{sc}2.1 ± 0.8 μA/cm^2). Interestingly, the combined treatment, TEZA+ELE, strongly increased the Cl^- secretion in A559T-colonoids monolayers (ΔI_{sc}13 ± 6 μA/cm^2), reaching 11% of WT CFTR function. These results suggest the important role of ELE in stabilizing A559T-CFTR through its interaction with the nucleotide binding domain 1 of CFTR. ELE was found to increase the amount of mature F508del-CFTR on cell-surface compared to TEZA (Keating, D. et al., 2018). Next, we will evaluate if the synergic work of the two CFTR correctors is needed to stabilize A559T-CFTR or if ELE alone is enough in restoring function to mutant A559T-CFTR due to its dual, corrector and potentiator, activities (Laselva et al.,2020;Veit G et al.,2021).

Conclusion: We showed a relevant response to ELE+TEZA in rectal organoids and NBEC with CFTR genotype A559T/A559. This could provide a rationale for treating this patient with IVA+TEZA+ELE that is in clinical use for patients carrying the F508del variant that falls in the same class II as A599T.

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Improved CFTR dependent sweating in people with cystic fibrosis treated with different combinations of CFTR modulators

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Background: Novel personalized therapies become prevalent in the clinical practice and new methods to assess their effects are needed. We previously applied the Optical Beta Adrenergic Sweat Test (OBAST) (Bergamini et al., 2018) to assess the CFTR protein function in cystic fibrosis (CF) patients before and during treatment with the combination of CFTR modulators ivacaftor+lumacaftor (IVA+LUMA) (Treggiari et al., 2021). According to our previous prediction based on multilinear regression analysis and data obtained in CF patients treated with CFTR targeted drugs we compared two different outcomes of this test: the single sweat droplets number (SDN) induced by microinjection of a beta-adrenergic cocktail with the ratio of sweat rate induced by the beta-adrenergic Cocktail (C) versus sweat rate induced by Methacholine (M) as cholinergic stimulus (C/M ratio).

Methods: Two intradermal microinjections (100ul) were performed in the forearm in order to induce first CFTR-independent sweat secretion by methacholine as a cholinergic stimulus, and then CFTR-dependent sweat secretion by a beta-adrenergic cocktail consisting of aminophylline and isoprenaline, also atropine was included for inhibiting the cholinergic CFTR-independent pathway. 32 CF patients were tested before and during treatment with LUMA or IVA+tezacaftor (TEZA) or IVA+TEZA+elexacaftor (ELE) for a period of time up to one year. Images of the single droplets formed on the forearm in an oil layer containing a water-soluble dye (erioglaucine disodium, Sigma) were acquired by a camera and then counted and measured at the end of both C and M phases, induced by Cocktail for 30 min and by Methacholine for 10 min, respectively. Both SDN and C/M ratio were determined and then compared. We considered clinical data of these patients such as the Body Mass Index (BMI), sweat chloride, Forced Expiratory Volume 1 (FEV1) and Nasal Potential Difference (NPD) measurements.

Results: We observed a statistically significant increase of SDN in these 32 CF patients with median value 13.5 (min-max -52.0 - 80.7; p-value = 0.006). We calculated a statistically significant C/M ratio median difference = 0.008 (min-max -0.58 – 0.83; p=0.02). Similar results were obtained in the subgroup of patients F508del +/+ (p=0.04 for both SDN and C/M ratio, n=11). Considering the subset of 21 patients carrying only one copy of F508del variant with data available before treatment and after six months we calculated a median increase of 12 SDN (min-max - 35 – 91, p=0.035), whilst no significant difference (p=0.3) was detected by using the C/M ratio (difference =0, min-max -0.5 – 0.4). Improvements of CFTR function measured by SDN, C/M ratio, sweat Cl were higher during treatment with IVA+TEZA+ELE in comparison with IVA+TEZA or IVA+LUMA in single CF patients when different treatment were applied in single CF patients, consistently with lung function and/or BMI and/or NPD changes.

Conclusions: Our findings show a significant improvement of CFTR function during treatment with different combinations of CFTR modulators and the improved ability to monitor this effect by using the simplified outcome SDN of OBAST test.

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Use of computer simulations to investigate ivacaftor-induced conformational changes in CFTR

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Treatment of cystic fibrosis has been transformed by orally administered drugs that directly target the cystic fibrosis transmembrane conductance regulator (CFTR). Ivacaftor, the first clinically approved CFTR potentiator, binds CFTR in a cleft formed by three transmembrane segments at the lipid-protein interface, which includes the unstructured region of transmembrane segment 8[1,2]. However, the conformational changes initiated by ivacaftor binding to CFTR remain largely unknown. Here, we investigate how the ivacaftor-binding site is allosterically coupled to functionally important regions of wild-type CFTR.

We used an emerging computational approach named dynamical-nonequilibrium molecular dynamics (D-NEMD) simulations[3]. This approach combines molecular dynamics (MD) simulations under equilibrium and nonequilibrium conditions to map the evolving structural response of a protein to the removal of a ligand from its binding site. Using molecular models based on the cryo-EM structure of ivacaftor complexed with phosphorylated, ATP-bound human CFTR (PDB ID: 6O2P)[2], which lack the R domain, we conducted extensive equilibrium MD simulations (five replicate 500 ns simulations, totalling 2.5 μs) and a large set of 410 short (5 ns) nonequilibrium simulations to identify conformational changes in CFTR elicited by the removal of ivacaftor from its binding site.

Our simulations demonstrated that the structural changes induced by ivacaftor removal started in the ligand-binding pocket, namely transmembrane segments 4, 5 and 8. Subsequently, the structural changes were gradually transmitted both upwards towards the extracellular vestibule of the CFTR pore and downwards through neighbouring transmembrane segments, ultimately reaching the ATP-binding sites located at the dimer interface of the nucleotide-binding domains.

Our work reveals how ivacaftor-induced structural changes are propagated from the ligand-binding site to the ATP-binding sites and the channel pore. We will use this approach to understand how ivacaftor restores function to CFTR proteins with gating mutations to inform the rational development of structure-guided therapies for cystic fibrosis.

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References:

1. Yeh et al. J Gen Physiol. 2019; 151:912-928.

2. Liu et al. Science. 2019; 364:1184-1188.

3. Oliveira et al. Eur Phys J B. 2021; 94:144.

P51

Intracellular delivery of stabilizing nanobodies to rescue CFTR mutants

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Cystic fibrosis is caused by mutation of cystic fibrosis transmembrane conductance regulator (CFTR) that is important for proper fluid balance across epithelia. Class II mutations, such as F508del, impairs CFTR stability and trafficking towards the plasma membrane. It was shown that F508del-CFTR is thermodynamically destabilized and NBD1 stabilization is necessary to overcoming the CFTR defect. However, the approved molecules, even the most recent Trikafta, are unable to thermally stabilized the mutant CFTR. Obviously, an improvement of the current treatment remains highly desirable. In this context, we have developed NBD1-stabilizing nanobodies in order to improve the current CF therapies. We have shown that plasmid transfection of our nanobodies restore maturation, expression and activity of F508del-CFTR synergically with the approved correctors leading to a wt-like behavior. In order to translate our results to therapeutics we explore different intracellular delivery methods. The first approach will make use modified nanobodies with cell-penetrating peptides (CPP) developed to allow direct protein delivery inside the cytoplasm. The second approach is based on recent progress of mRNA-based medicines, we propose to use mRNA as a therapeutic modality to provide stabilizing nanobodies to target tissues; more specifically the airways. We designed a mRNA encoding for one of our stabilizing nanobody. We show that our nanobody-mRNA promote maturation (detected by Western blot) and cell-surface expression of F508del-CFTR (detected by flow cytometry). This effect is highly synergistic with that of approved correctors, including Trikafta. In therapeutic perspective, we will develop a lung-targeting lipidic nanoparticules formulation to allow the penetration of our mRNA to pulmonary tissue by nebulization. Our work will provide novel therapeutic routes to treat cystic fibrosis.

P52

Characterization of the novel 1210-2A>G variant by means of patient-derived nasal epithelial cells: molecular and functional analysis of CFTR mRNA and protein

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Mutations of the *CFTR* gene affect the encoded protein through a variety of molecular mechanisms, leading to different functional defects. Until now,more than 2000 mutations have been identified. Some variants are well studied while others have not been yet characterized, also due to their low frequency.

Understanding the functional consequence of rare CFTR variants is essential for the adoption of precision therapeutic approaches for CF.

In the frame of a project aimed to characterize rare CFTR mutations, we focused on a patient compound heterozygous for the novel 1210-2A>G substitution, and a well-known large deletion, CFTRdele 17a, 17b, 18. The 1210-2A>G affects the canonical acceptor splicing site of the CFTR exon 10, for which different causative variants have been already reported.

Patient's cells from nasal brushing were cultured, expanded and reprogrammed to generate differentiated epithelia. Then CFTR function was evaluated in Ussing chamber upon treatment with different CFTR modulators. Cells were then harvested for RNA extraction to allow study of the CFTR mRNA.

This analysis demonstrated that the 1210-2A>G variant causes the skipping of exon 10, an event that can also occur in wild-type *CFTR* with different extent. The different proportion of transcripts with exon 10 included or skipped is affected by the presence of an adjacent Poly-T and Poly-TG polymorphic sequences with different alleles some of which behaving as intragenic modifiers or variants predisposing to CFTR-related disorders.

The genotype of the patient at this *locus* ([c.1210-34_1210-6TG(10)T(7); c.1210-34_1210-6TG(10)T(9)], legacy name TG10T7; TG10T9) is reported to favor the wild-type processing of the mRNA, therefore the skipping of exon 10 is to be entirely ascribed to the presence of the 1210-2A>G variant.

We further demonstrated this hypothesis by applying a minigene approach in a heterologous system. To this purpose we amplified the exon 10 and flanking sequences from patients' genomic DNA, in this way we could isolate and study the wild-type allele versus the 1210-2A>G substitution with the same configuration respect to the TG10T7; TG10T9 polymorphism as *in vivo*. The variant abolished the canonical splice site and causes the skipping of the whole exon 10.

The transcripts analysis in HNEC from the patient allowed to verify that the second allele, CFTRdele 17a, 17b, 18, leads to the skipping of exons 19,20 and 21 with the maintenance of the reading frame.

The functional study by means of short-circuit current analysis on patient-derived nasal epithelia highlighted some modest residual activity that however was not significantly augmented after treatment with pharmacological agents.

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P53

Clinical consequences and functional impact of the rare S737F CFTR variant

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Cystic Fibrosis (CF) is caused by mutations in the CFTR gene encoding the CFTR channel and is characterized by a high allelic heterogeneity. Some variants are well studied and have been found to act by different mechanisms, as in the case of F508del, the most frequent in CF patients, causing defective maturation and gating. To date, pathogenicity has been demonstrated for approx. 400 CFTR variants out of more than 2000 described. However, several variants have not been characterized yet, also due to their low frequency and many are still referred as of uncertain significance.

S737F is a CFTR substitution, typical of Tuscany region, Italy. Serine 737 is one of the phosphorylation sites in the regulatory domain of CFTR, involved in regulation of protein function and expression at the plasma membrane. This variant is associated to hypochloremic alkalosis in childhood and mild CF phenotype in teenage years, however no clinical information after a longer follow up or functional analysis have been reported.

We retrospectively investigated the clinical features of a group of ten individuals carrying at least one S737F CFTR variant, having at first evaluation CF (n.1), or CRMS/CFSPID (n.6) or inconclusive diagnosis (n.3). At study end, five asymptomatic individuals were diagnosed with CF, 2 were still CRMS/CFSPID, while three subjects did not meet all criteria to support a CF diagnosis neither had the single-organ involvement of a CFTR-RD. As recently suggested, CFTR dysfunction compatible with CFTR-RD is defined by evidence of in vivo or ex vivo CFTR dysfunction in the CFTR-RD range in at least 2 different CFTR functional tests [sweat test, nasal potential difference (NPD) and intestinal current measurement (ICM)]. NPD and ICM are however not so easily accessible. Ex vivo studies performed on patient-derived nasal cells can be a non-invasive tool that may contribute to classify these patients.

We characterized in detail the impact of S737F mutation on CFTR expression and function. By ex vivo analyses, performed on patient-derived nasal epithelia, we evaluated the chloride secretion mediated by the variant protein as well as its responsiveness to CFTR modulators. Finally, the in vitro studies on the S737F-CFTR expressed in a heterologous expression system allowed us to further characterize the function and expression pattern of the variant protein in a neutral cell background.

The patient compound heterozygous for the S737F and dele22-24 variants showed a reduced CFTR activity (10.1 ± 1.1 μA), which points towards a diagnosis of CFTR-RD, given that functional threshold for CFTR-RD seems to be between 10% and 30% of the normal value. Higher total CFTR activity was found for the other 2 subjects, homozygous for the S737F variant (24.2 ± 3.6 μA) and heterozygous for the S737F (20.4 ± 3.8 μA). Based on these data we could differentiate the follow up of the latter two, avoiding hyper medicalization.

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Elexacaftor/Tezacaftor/Ivacaftor triple therapy corrects function of CFTR rare variants

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Objectives: In Europe the triple combination Elexacaftor/Tezacaftor/Ivacaftor (ETI) is accessible only for patients carrying p.Phe508del (F508del) mutation on at least one allele, while the Food and Drug Administration (FDA) labelled ETI for patients carrying 177 additional CFTR variants. Considering that a number of severe patients carrying rare variants may benefit from ETI, a compassionate therapy program was launched in France. Those patients were invited to perform a nasal brushing to correlate the clinical efficacy of ETI with the correction of CFTR activity evaluated in vitro in Human nasal epithelial (HNE) cell cultures.

Methods: CFTR activity correction was measured by short circuit current in HNE cultures at basal state (DMSO incubation) and after ETI incubation (48h) and expressed as % of normal CFTR activity (CFTR WT) after sequential addition of Forskolin and Inh-172 (ΔIsc ETI/DMSO %WT).

Results: Eleven patients with CF carried variants eligible to ETI according to the FDA list: I601F, G85E, S492F, M1101K, R3479P, R74W, and H1085R. Twenty eight carried variants not listed by FDA: large deletions (CFTRdele19 and CFTRdele3-10;14b-16); nonsense variants (Q493X, G542X, R553X, E585X, K710X, W1063X E1104X, R1162X, W1282X); a variant anticipated to inhibit translation initiation (M1T); variants introducing a frameshift and predicted to generate a Premature Termination Codon (357delC, 1078delT, 2183AA>G, 3659delC, 4271delC); splicing mutations (711+1G>T, 1525-1G>A, 1717-1G>A, 4374+1G>A, 2789+5G>A, 4096-3C>G); missense mutations shown to generate a severely misfolded protein (I507del, N1303K, R334W, R1066C, L558S) or whose mechanisms have not been yet documented (Q552P).

ETI significantly increased CFTR activity of FDA approved CFTR variants. We point out the correction by ETI of additional non FDA variants, including N1303K, R334W, R1066C, Q552P and terminal splicing variants (4374+1G>A, 4096-3C>G). Correction of $Isc_{ETI/DMSO}$ %WT was significantly correlated to the change in ppFEV 1 ($p < 0.0001$), and of the sweat test ($p < 0.0001$). G85E, R74W and Q552P were rescued more efficiently by other CFTR modulator combination than by ETI.

Conclusion: Primary nasal epithelial cells hold promise for expanding CFTR modulator treatment in patients carrying rare mutants. Additional variants than those listed by FDA should be discussed for ETI indication.

P56

Correction of CF-causing mutations L227R and N1303K by prime editing

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Objectives: Gene therapy for cystic fibrosis (CF) has been pursued since the early 90's and focused on a gene addition approach, which adds a *CFTR* cDNA to restore CFTR function. Gene correction on the other hand, allows to restore mutations in patients'chromosomes, thereby preserving endogenous gene expression and regulation and possibly providing a permanent cure. We hypothesized that prime editing (PE), one of the most recent gene editing technologies, could be leveraged to permanently and precisely correct drug-refractory CF-causing mutations, such as L227R (c.680 T>G) and N1303K (c.3909 C>G).

Methods: To obtain optimal editing efficiencies, we screened over >20 guide combinations for each mutation via transfection in HEK293T cells overexpressing mutant *CFTR*. After three days, genomic DNA was extracted and evaluated by Sanger sequencing. Gene editing efficiency was also verified at protein level to detect restored CFTR glycosylation by Western blot, plasma membrane (PM) localization by confocal microscopy/flow cytometry and ion channel function by HS-YFP quenching. Additionally, we investigated the use of engineered prime editing guides (epegRNAs) that contain a structural terminal motif capable of protecting the RNA against intracellular degradation. To validate our approach in a more relevant and translational model, we delivered the PE enzyme together with the required guides to 16HBE airway epithelial cells and patient-derived rectal organoids via lentiviral vectors (LV). In order to determine functional correction in the organoids, we performed forskolin induced swelling (FIS) and developed an AI-based algorithm to identify identify the percentage of FIS-responsive organoids. In 16HBE-N1303K, HS-YFP quenching was used to evaluate CFTR function. Finally, targeted deep sequencing was performed to determine potential off-target edits throughout the genome.

Results: Use of optimized pegRNAs and PE approaches allowed to achieve significant genetic and functional correction in HEK293T. Sanger sequencing indicated 23 3,8% DNA correction for L227R and N1303K respectively (mean + SEM). Use of epegRNA variants allowed to increase editing efficiency 1.49 and 1.33-fold respectively.

LV delivery of PE components to intestinal organoids, resulted in a functional recovery of CFTR activity mediated through correction of the endogenous *CFTR* gene. For N1303K and L227R respectively, FIS responses up to 45% and 85% were measured using the AI-based analysis. In preliminary experiments, delivery of PE to 16HBE-N1303K airway epithelial cells led to 30% DNA correction and a corresponding functional response of 27% relative to wild type 16HBE on the HS-YFP quenching assay. Targeted deep sequencing did not reveal any significant off-target edits.

Conclusion: We have shown that both the L227R and N1303K CF-causing mutations can be safely corrected by PE leading to a functional recovery of CFTR in cell lines and patient-derived rectal organoids. Ongoing work focuses on tailoring a translational delivery vehicle for the gene editing machinery.

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P57
Pan-ethnic characterization of CFTR variants

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Introduction: In cystic fibrosis (CF), small molecule therapies targeting the basic CFTR defects have shown clinical benefits, but therapeutic responses are largely dependent on the patients' *CFTR* genotype. Although CF is most prevalent in people with European ancestry, the distribution of dominant *CFTR* or CF-causing variants vary across ancestries. Thus, characterizing the broad spectrum of pathogenic and non-pathogenic *CFTR* variants across ancestries is critical for revolutionizing molecular diagnoses of CF.

Methods: We analyzed 454,857 whole-exome sequences generated by UK Biobank (UKBB) to characterize the diversity of *CFTR* variants across ancestries. We used the ancestry assignment done by the pan-UKBB to investigate the frequency of *CFTR* variants in Africans, American, Central South Asian, East Asian, European, and Middle Eastern populations. We then performed an overlap analysis to decipher ancestry specific *CFTR* variants. CF-causing variants were then annotated using the CFTR2 database before populating the frequency distribution across ancestries.

Results: Overall, we detected over 4,000 *CFTR* variants from all whole exome sequences analyzed in this study. Many of these variants have never been reported in CF. The highest number of *CFTR* variants were detected in Europeans [n=3,192] while American group had the least number of *CFTR* variants [n=1507]. We found several variants specific to each ancestry, with Europeans having most of the unique variants [n=2212] while the American group has the least number of unique *CFTR* variants [n=23]. F508del was the most prevalent CF-causing variant found in all ancestries, except in East Asia, where V520F was the most prevalent. Participants undiagnosed as CF but harboring two CF-causing variants (n>10) reported phenotypes significantly (adjusted $p<0.05$) associated with CF pulmonary phenotypes.

Conclusion: Most CF-causing variants have been characterized by investigating European populations. The identification of several unique uncharacterized variants in other ancestries warrants the need for further studies to delineate their functional relevance. The presentation of classical CF phenotypes seen in some non-CF diagnosed participants, with more than one CF-causing variants, indicates they may benefit from current CFTR therapies.

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P58

A precise adenine base editor corrects W1282X-CFTR without bystander effects

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W1282X (c.3846G>A) is the sixth most common cystic fibrosis (CF)-causing variant. This nonsense mutation generates a premature termination codon, which leads to transcript degradation by nonsense-mediated decay, and, consequently, no full-length functional CFTR protein is produced. Even though four CFTR modulators have been approved for clinical use, people with CF (PwCF) carrying nonsense mutations do not benefit from any of these targeted therapies. Adenine base editing (ABE) has attracted interest as it can correct certain nonsense mutations without creating a double-stranded break in DNA, being thus potentially safer for therapeutic use than the traditional approach using CRISPR/Cas9 nuclease. ABE uses a fusion of a Cas9 nickase and a synthetic TadA enzyme modified to convert any adenine (A) into guanine (G) in an editing window spanning nucleotides 4 to 7 of the spacer. Several research groups including ours have previously reported efficient A>G editing at position A6 which corrects the W1282X variant by ABE [1]. However, a high level of bystander editing was observed at the adjacent adenines, particularly A7.

Following previous observations that splitting the Cas9 protein into two halves would minimize the duration of nuclease activity and reduce off-target/bystander effects, we designed two new fusion proteins - NLS-FKBP12-Cas9(C)-NLS and TadA-NES-Cas9(N)-FRB - and developed a novel ABE (hereinafter split-ABE), which should only dimerise and function in the presence of rapamycin. To test the split-ABE, we generated a W1282X version of the BCi-NS1.1 cell line, an immortalized line that can differentiate into all known lung cell types. These cells were electroporated with the split-ABE or SpRY-ABE (non-split control) plasmids and treated with rapamycin or DMSO (vehicle control). The DNA was extracted from cells 72h after treatment and the target region was amplified by PCR. The editing efficiency was measured by next-generation sequencing (Amplicon-EZ, Azenta).

Using the control SpRY-ABE we detected on-target editing at A6 in 5.1% of alleles but with a high level of bystander editing at position A7 of 7%. Whilst on-target editing with the split-ABE in the presence of 200 nM rapamycin was only detected in 2.1% of alleles, almost no bystander editing at A7 was observed (≤0.5%).

In summary, we showed the development of an improved version of the already existing ABE, which can successfully correct W1282X-CFTR while substantially reducing bystander edits. The fact that the split-ABE can be regulated by a small molecule drug gives us temporal control of the editing. Currently, we are developing a system for cell type-specific expression of the split-ABE, which will allow us to have spatial control of editing. The longer-term goal is to understand which cell types, and how many of them, need to be corrected to restore CFTR function to therapeutic levels using our BCi W1282X-CFTR model.

[1] Mention K et al., 2023 (Submitted to Hum. Mol. Genet.)

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Targeted repair of cystic fibrosis mutations across an entire exon using Cas9 and a single guide RNA and donor template

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Precision gene editing using CRISPR is a promising therapeutic approach to treat genetic disorders, such as cystic fibrosis (CF). We, and others, have demonstrated single CF-causing mutations can be corrected via homology-directed repair (HDR) using Cas9/gRNA and a DNA donor template[1]. In genetic disorders caused by multiple mutations, such as CF, it would be beneficial to correct several different mutations located in the same exon with a single donor template/gRNA combination. However, CRISPR HDR strategies are limited by the efficiency of precision repair beyond a very short distance from the Cas9/gRNA target site.

The 95 bp exon 12 of the CFTR gene contains 18 different CF-causing variants, including the second most common CF mutation, G542X, and the second most common splice variant, c.1585-1G>A. Even with the short 39 bp distance between these two mutations, it is currently not feasible to repair both mutations with a single donor template/gRNA combination by HDR. This is because donor templates must contain regions of homology with the target DNA to facilitate the formation of a so-called D-loop in order to introduce site-specific changes by HDR. However, homologous sequences within the donor template can also induce D-loop collapse, reducing the length of the HDR editing window[2]. Here, we aim to circumvent D-loop collapse through introduction of regions of reduced-homology within the donor template repair track, but still encoding the same amino acid sequence. Using this approach, we aim to generate a single donor template and gRNA capable of repairing all CF-causing mutations across exon 12.

To demonstrate extension of the HDR editing window, we are initially focusing on developing a single gRNA and donor template to correct three exon 12 mutations, namely G542X, located within exon 12, and two splice mutations at the 5' and 3' exon-intron boundaries, c.1585-1G>A and c.1679+1G>A. To this end, we have designed and tested several gRNAs targeted to intronic regions within 50 bp of exon 12. Suitable gRNAs were identified based on indel frequency as assessed by amplification and sequencing of the target region. As a CFTR model, we are using HEK293 Flp-In cells containing the expression mini-gene (EMG) CFTR with the respective variant. To generate these cell lines, we first altered the CFTR wild-type cDNA EMG construct to incorporate exon 12 flanking introns using gBlocks and restriction digestion cloning. Site-directed mutagenesis of the resulting construct produced mutant EMG constructs which were then co-transfected with Flp recombinase to generate stable mutant CFTR cell lines. The efficiency of HDR extension with the selected gRNAs and different modified donor templates will be tested in these models to establish the viability of the editing approach. Thereafter, the three exon 12 mutations will be introduced separately into the BCi-NS1.1 human airway epithelial cell line to create physiologically relevant models of CFTR, thereby facilitating functional assessment of correction mediated by gRNAs and modified donor templates.

1. Santos et al. (2022) *J Cyst Fibros* 21: 181-187

2. Byrne et al. (2015) *Nucleic Acids Res* 43: e21

P60

Antisense oligonucleotides direct endogenous ADARs to recode a premature termination codon in CFTR mRNA

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Introduction: Cystic fibrosis (CF) is an autosomal recessive disease, caused by mutations affecting the CFTR chloride channel. Approximately 10% of CF patients worldwide harbor nonsense (or stop) mutations in the CFTR gene. Nonsense mutations create a premature stop codon (PTC) in the mRNA leading to the premature arrest of CFTR protein translation and the production of a truncated and non-functional protein.

Objective: Despite the intensive research in the field, patients carrying stop mutations cannot benefit from approved therapies yet. Hence, we are investigating an approach based on sequence-specific RNA editing that can rescue CFTR stop mutations.

Methods: This approach employs specific antisense RNA oligonucleotides (ASOs) made up by an ADAR-recognizing sequence and another part complementary to the target mutated region of the CFTR mRNA apart from a cytidine-adenosine mismatch exactly where a UGA PTC lies. The A:C mismatch induces the recruitment of the endogenous Adenosine Deaminases Acting on RNA (ADARs) that catalyze the conversion of the target adenosine of UGA stop codon into inosine. The incorporated inosine will be decoded as guanosine by ribosomes allowing the translation process to continue and restore the CFTR full-length protein.

Results: Our results show that the transfection of ASOs in human bronchial epithelial cells harboring the W1282X or the G542X stop mutations restores the CFTR protein on the plasma membrane and increases CFTR transcript expression compared to the untransfected mutated cells.

Conclusion: Finally, these results could represent an appealing therapeutic strategy to correct the nonsense mutations causing cystic fibrosis.

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The Cells Below: tackling lung basal cells' encasement with VP22 fusion proteins

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Gene editing of lung basal cells (BC) has the potential to permanently correct any cystic fibrosis (CF) causing mutation in the CFTR gene. Whilst surface epithelial cells (SEC) can be readily transfected with DNA or RNA molecules encoding gene editing constructs, the composition of the lung pseudostratified epithelium causes an impediment to the direct targeting of BC, as efficient delivery of DNA or RNA is mainly limited to SEC. Considering this, is it then possible to deliver a gene editing cargo to the SEC that could then transfer its encoded products to the nuclei of cells below? Inspired by previous studies[1] using Herpes simplex virus 1 VP22 cell penetrating peptide (CPP), here we describe the construction of VP22-GFP fusion proteins and assess the intercellular trafficking and nuclear localisation capacity of VP22-GFP constructs in HEK293T cells and in a pseudostratified mucociliary epithelium cell model differentiated from BCi-NS1.1[2]. The ultimate goal is to assess if this CPP can shuttle gene editing complexes to the nuclei of BC in a pseudostratified epithelium and correct CF-causing mutations.

To create pVP22-GFP expression vectors to test this hypothesis, two gBlocks encoding for a VP22 sequence with or without a Kozak consensus sequence were designed and cloned in-frame upstream of GFP under control of a CMV promotor in an expression vector (IDTdna.com). HEK293T cells transfected with either of the pVP22-GFP vectors or the pCMV-GFP control vector displayed high levels of GFP expression when measured by flow cytometry 48 hours post-transfection. However, a distinct nuclear localisation pattern was observed in cells transfected with pVP22-GFP plasmids, while the pCMV-GFP control displayed high levels of GFP throughout the entire cell.

To determine if the VP22-GFP fusion protein had the ability spread to neighbouring cells, either pVP22-GFP or pCMV-GFP transfected cells were added to a flask containing adherent non-transfected HEK293T cells at a seeding density of 2:5, then imaged 48 hours later. HEK293T cells co-cultured with pVP22-GFP transfected cells showed detectable nuclear GFP fluorescence which supports the hypothesis that VP22-GFP fusion proteins are capable of intercellular trafficking and nuclear targeting. In contrast, HEK293T cells co-cultured with pCMV-GFP transfected cells showed no detectable nuclear localisation or intercellular trafficking.

The focus is now to determine the potential of the VP22-CPP to target GFP into the BC located at the base of the differentiated BCi-NS1.1 cell model by confocal microscopy, which we routinely differentiate into multipotent progenitor populations of the airway epithelium using 28 days-differentiated air liquid interface cultures, and then assess the targeting ability of VP22 CPP fused to gene editing constructs.

1. Elliott, G. & O'Hare, P. Intercellular trafficking of VP22-GFP fusion proteins. Gene Therapy (1999).
2. Walters, M. S. et al. Generation of a human airway epithelium derived basal cell line with multipotent differentiation capacity. Respir Res 14, (2013).

P62

Rescuing G542X by Adenine Base Editing: a guide to restore function

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G542X is the 2nd most common CF-causing mutation. The premature termination codon (TGA) and the consequent nonsense mediated decay prevent CFTR protein synthesis. As no protein is produced, G542X is untreatable with current modulators.

Adenine Base Editing (ABE) is a CRISPR-based approach that is widely recognised as safe and efficient to edit CF mutations. Whilst ABE cannot precisely repair G542X, it can be used to get rid of the stop codon by converting G542X into G542R, a CFTR variant which has been shown to retain about 25% of WT activity (Xue et al., 2017). Specifically, ABE can be used to edit the A on the opposite strand, at the first position of the TGA codon, into a G, thus converting the TGA (stop) codon into CGA (Arg).

Our initial aims were to

(i) test the efficiency of our design of a single-guide-RNA (sgRNA) to edit G542X into G542R,

(ii) assess the level of rescue of CFTR expression and function following the editing, and

(iii) evaluate the extent to which G542R activity could be boosted even further by modulators.

The editing was first tested in Fisher Rat Thyroid (FRT) cells, the CF model carrying CFTR-cDNA that has been used to identify modulators. The sgRNA and the adenine base editor “ABE8e-NG” were delivered to the cells either as plasmids, by Lipofectamine 3000, or as ribonucleoproteins (RNPs), that we encapsulated in engineered Virus-Like Particles (eVLPs - Banskota et al., 2022). To assess editing, 72 hours post transfection/transduction, genomic DNA was extracted and PCR-amplified. Sanger sequencing showed that ABE by plasmids successfully edited G542X into G542R with 17% efficiency, while ABE delivered by eVLPs yielded 14% of editing. Using Western Blot, we also detected CFTR bands B and C, showing partial protein synthesis restoration by both methods of delivery. Finally, Short Circuit Current (*I*sc) on the eVLPs-treated cells revealed 4.2% of WT CFTR activity. Interestingly, this value was substantially increased by the addition of 5μM VX-809: it almost doubled the activity of CFTR, reaching 7.3% of WT levels.

If we extrapolate our *I*sc data and assume a linear relationship, this suggests that, if 100% of editing could be obtained, CFTR activity rescue would reach 30% of WT CFTR, which aligns with the aforementioned study (Xue et al., 2017). Activity could even go up to 52% of WT with VX-809, or possibly even higher with other modulators.

Our focus is now to:

(i) optimise editing levels,

(ii) assess the action of the most recent modulators on a clonal population of edited (possibly primary) cells,

(iii) correlate editing levels with functional activity.

References:

Banskota, Samagya et al. “Engineered virus-like particles for efficient in vivo delivery of therapeutic proteins.” Cell vol. 185,2 (2022): 250-265.e16. doi:10.1016/j.cell.2021.12.021

Xue, Xiaojiao et al. “Identification of the amino acids inserted during suppression of CFTR nonsense mutations and determination of their functional consequences.” Human molecular genetics vol. 26,16 (2017): 3116-3129. doi:10.1093/hmg/ddx196

P63

Nonsense mutations suppression in cystic fibrosis model systems by translational readthrough-inducing drugs (TRIDs)

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Background: 10% of cystic fibrosis (CF) patients are affected by a nonsense mutation in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene (1). The consequence is the formation of a premature termination codon (PTC) in the mRNA and the production of a truncated protein, rapidly degraded (2).

Among different therapeutic approaches, suppression therapy, by Translational Readthrough Inducing Drugs (TRIDs), is the most promising strategy against this alteration. It is aimed to skip the presence of the PTC, by forcing the insertion of a near-cognate tRNA (nc-tRNA), allowing to the production of a full-length and possibly functional protein (3).

In the last decade, three new TRIDs, named NV848, NV914 and NV930 [Italian Patent N.102017000134511, European Patent N.3713934, US Patent N.11,203,578], have been optimized and tested in different nonsense cell models, showing high readthrough activity on the PTCs. The molecules were also validated in vitro and in vivo for their safety (4, 5).

Objectives and methods: This study aims to examine different aspects of NV compounds from molecular and physiological points of view.

The first purpose was to investigate the biodistribution of one of the three compounds, NV848, in target organs interested in CF (lungs, intestines, pancreas, plasma, kidney, brain). The analyses were performed at different time-points by HPLC, after oral administration of 60 mg/Kg of NV848 molecule. This experiment was necessary and preventive to the following step, that was to evaluate CFTR expression in $CFTR^{G542X/G542X}$ murine model after oral administration of 60 mg/Kg of NV848 molecule.

Finally, to explore the possible biological target and mechanism of action (MoA) of the three NV compounds, the last objective was to study the possible interaction between the NV molecules and the FTSJ1 methyltransferase, responsible for tryptophane tRNA modifications (6).

Results and conclusions: Biodistribution results showed that the NV848 molecule has reached the organs of interest and in particular lungs, pancreas, and intestines.

Moreover, the administration of the NV848 molecule to $CFTR^{G542X/G542X}$ mice has evidenced a sensible increase of the CFTR mRNA and the rescue of CFTR protein expression in the lung of the treated mice.

In addition, the experiments performed to study the biological target of the NV molecules have suggested that the NV914 molecule could interact with the FTSJ1 methyltransferase during the readthrough process.

In conclusion, our data are promising and pave the way to the development of the future steps in order to optimize the suppression therapy by our NV TRIDs.

References:

(1) Kerem E. Expert Opin Investig Drugs(2020)Dec;29(12):1347-1354..doi: 10.1080/13543784.2020.1828862.

(2) Zheng-Hong Q. Basic Science(2019).DOI:https://doi.org/10.1007/978-981-15-0602-4.

(3) Dabrowski M. RNA Biol(2015);12(9):950-8.doi: 10.1080/15476286.2015.1068497.

(4) Pibiri I. Int J Mol Sci(2020)Sep 3;21(17):6420.doi: 10.3390/ijms21176420.

(5) Corrao F. Biomed Pharmacother(2022)Dec;156:113886.doi: 10.1016/j.biopha.2022.113886.

(6) Trzaska C. Nat Commun(2020)Mar 20;11(1):1509. doi: 10.1038/s41467-020-15140-z.

P64

Correcting two CF-causing mutations with a single epegRNA by PRIME editing

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Precision repair of mutations by CRISPR is a very strong candidate for development of treatments for genetic diseases. Typically, CRISPR based methods can correct one mutation at a time which makes it more challenging to develop a genetic treatment for diseases such as CF that are caused by multiple mutations. CRISPR PRIME, a recently developed CRISPR based method allows us to correct clustered multiple mutations.

Here, we report correction of two CF-causing, nonsense mutations that are in close proximity, R1158X and R1162X with a single pegRNA. In addition to targeting multiple mutations, CRISPR PRIME has two main advantages for clinical development over other CRISPR methods. It does not introduce double-stranded breaks in the genome and does not require a donor DNA template. It has only two components; an engineered reverse transcriptase (RT) fused to a Cas9 nickase (the PE2 protein) and a multifunctional prime editing guide RNA (pegRNA). The pegRNA contains both a spacer sequence to target DNA and a 3' extension that encodes the desired edit. The PE2/pegRNA complex binds to the target region and creates a nick three nucleotides upstream of the protospacer adjacent motif (PAM) liberating a 3'flap. This flap hybridizes with the primer binding site (PBS) at the 3' extension of the pegRNA and forms an RNA/DNA hybrid that serves as the primer site for new DNA synthesis which is catalysed by the RT. This initial prime editing system has been modified by several groups to increase editing outcomes. PE3, PE4 and PE5 are among the enhanced systems; PE3 increases efficiency through nicking the un-edited strand with an additional nicking guide RNA, PE4 through repression of mismatch repair and PE5 through combination of the two systems. Another improvement to editing outcomes came from the modification of pegRNA structure to be more stable against RNA degradation (epegRNA).

Since the first step of PRIME editing is the nicking of target DNA by the guide RNA, we first tested efficiency of several guide RNAs that target the region. Spacer sequences corresponding to the two guides with highest activity were used to design epegRNAs with varying PBS and RT lengths to target both mutations at the same time. We tested these in two different Flp-In 293 CFTR Expression Mini-Gene (EMG) cell lines; one with R1158X, the other with R1162X mutation. Initial deep sequencing results showed that two of these epegRNAs that had the same spacer sequence could correct R1158X mutation on genomic DNA. When the same epegRNAs were tested in the R1162X cell line, they could also correct that mutation. We then confirmed these results on the mRNA level by deep sequencing. Using enhanced editing systems; PE3, 4 and 5, we were able to increase the editing efficiency up to 1.4% in a population of transfected cells. We are working on boosting the efficiency by modifying the epegRNAs to disrupt the PAM sequence and by using alternative more efficient PE2 protein structures. Overall, our results show for the first time that a single epegRNA can correct two CF-causing mutations.

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Identification of microRNAs associated with disease severity in cystic fibrosis siblings

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Cystic fibrosis (CF) is one of the most common autosomal recessive genetic disease with an estimated frequency of 1/3000 in Turkey. It is known that mutations detected in CFTR gene vary depending on ethnicity of the patients. Mutations in the Turkish population are heterogeneous and different from Europe. The frequency of F508del is detected as 28 % due to genetic heterogeneity. In addition, there are patients with different clinical severity despite having same CFTR mutation. The heterogeneity of disease severity among patients makes it difficult to establish genotype phenotype correlation. Therefore epigenetic factors such as microRNAs may affect and have a role in disease progression.

In this study, CF siblings with different clinical severity despite having the same mutation F508del (2 families with 2 siblings from each family) were determined. Clinical severity of siblings were determined according to number of lung infections, gastrointestinal system involvement and percentage of forced expiratory volume in 1 s (FEV1%) (predicted). miRNAs that show differential expression in nasal cells between severe and mild siblings were identified by using GeneChip miRNA 4.0 Array (Affymetrix). According to Transcriptome Analysis Console 2.0 program, hsa-miR-6732-5p (FC: 6.25), hsa-miR-6858-5p (FC: 1.91) were found to be upregulated, whereas hsa-miR-1180-3p (FC: -1.88), hsa-miR-34c-5p (FC: -.4.44), hsa-miR-34c-3p (FC: -16.22), hsa-miR-92b-3p (FC: -24.6), hsa-miR-449c-5p (FC: -54.69) were found to be downregulated in severe siblings compared to mild ($P < 0.05$).

The potential target genes of miRNAs were identified by using Targetscan and miRWalk 3.0. Genes that were related to cilia were obtained using SYSCILIA database. VENNY 2.1 was used to determine overlapping genes between potential target genes and cilia genes. DAVID database was used to perform Gene Ontology analysis. The top six significantly enriched Gene Ontology pathways were cilium assembly, ciliary body, cilium biogenesis/degradation, intraflagellar transport, immune system development and Wnt signaling pathway ($P < 0.05$).

Common miRNAs that cause clinical heterogeneity among siblings carrying the same mutation was identified for the first time in this study. After validation of the expression levels of miRNAs in larger patient sample size, a prognostic miRNA signature which will facilitate the early determination of the course of the disease will be detected. In addition, new mechanisms and pathways associated with CF will be elucidated.

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Correction of the CFTR 1717-1Ggt;A splicing mutation through CRISPR based technology

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The 1717-1G>A substitution is one of the most common Class 1 mutation, affecting approximately 1% (CFTR2 database) of cystic fibrosis (CF) patients. This mutation is characterized by a G to A conversion in intron 11, which disrupts a conserved AG dinucleotide at the 3' end acceptor splice site leading to exon 12 skipping of recognition of a newly generated cryptic splice site. Both altered splicing products generate a premature stop codon and subsequent lack of CFTR protein synthesis. The aim of this study is developing a "second generation" genome editing approach to correct the 1717-1G>A mutation through the most advanced CRISPR-Cas technology: base- and prime-editors.

To set up the CRISPR-Cas strategy we developed a 1717-1G>A HEK293 cell model mimicking the splicing defect. We obtained over 40% of correction using NG-ABE8e and NG-ABE8.20m base-editors through plasmid transfection. Nevertheless, high levels of bystander edits (secondary modifications associated with base editors) were associated with both ABEs, which resulted in unwanted modifications near the mutated nucleotide with potential detrimental effects in CFTR protein synthesis. As alternative strategy we tested the prime editing technology, resulting in an efficient correction over 40%, matched by a significant rescue splicing patter and membrane localization in the 1717-1G>A HEK293 model. However, due to low efficiency in installing the desired modification in the endogenous *CFTR* locus, an alternative strategy using a nuclease-based prime editor was explored, reaching editing efficiencies above 20%.

Overall, we obtained data showing that several CRISPR-Cas approaches can be exploited to repair the 1717-1G>A mutation, with prime editing technologies capable of providing precise modification over the bystander unwanted mutations introduced by the base-editors. Further optimization of the prime-editor nuclease based-strategy is ongoing to correct the mutation in patient-derived epithelial cells and organoids.

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Rescue of various deep intronic mutations by an oligonucleotides-based strategy

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Introduction: Several CFTR therapeutic strategies have been approved and the number of CFTR mutations targeted by therapeutic agents is increasing. Approximately 10%-20% of patients are not still eligible to CFTR modulators, particularly patients with splicing mutations (~12% of the reported CFTR mutations). We specifically focused on deep intronic mutations that stimulate the recognition of aberrant splicing patterns including the insertion of an intronic part in the mRNA sequence (cryptic exon). These splicing mutations generally lead to the formation of premature termination codons of translation. We proposed an approach of antisense modified oligonucleotides (AONs), to specifically target deep intronic mutations, through their binding on aberrant splicing sites of pre-mRNA. We designed AONs for 10 deep intronic mutations and, we assessed their efficacy to correctly regulate pre-mRNA splicing in bronchial cells and when possible in CF epitheliums.

Materials and methods: Minigene plasmids with the intronic sequence (400-600pb) encompassing either the normal or the mutation have been constructed for various intronic mutations including c.1680-883A>G, c.1680-886A>G, c.1680-877G>T, c.3874-4522A>G mutations. CFTR expression plasmids were constructed with the full length CFTR cDNA into which flanking intronic sequence of exons on either side of the cryptic exon (CE) and the region that includes CE were inserted. Chemically modified AONs were specifically designed for each deep intronic mutation, included in this study, and treatment with different concentrations were performed. Various cellular models have been used: Beas 2b for minigene assays and primary cultures from CF patients nasal cells cultured in monolayers or in epithelium (ALI, Air Liquid Interface). RNAs and proteins were extracted 24h and 48h post-transfection and analyzed (RT-PCR, fragments analysis or western blot). Efficacy of AONs has been assessed alone, in mixture, or in combination to CFTR modulators and chloride measurements, 48h post-transfection, were carried out, when possible, by using Ussing chamber system.

Results: Minigene assays showed that AONs effectively block cryptic exon inclusion for all intronic mutations tested. Different concentrations (from 20nM to 1uM) and timing have been assessed. Treatment with AONs induced a strong corrective effect for the c.1680-883A>G and the c.1680-886A>G mutations (up to 70% of normal mRNA) at low concentration (50nM) in bronchial cell lines and in primary nasal cultures. Transfection of CFTR expression plasmids containing the c.3874-4522G with AONs led to an increase in normal splicing of CFTR transcripts and the appearance of full length CFTR protein in bronchial epithelial cells. First results obtained in ALI epithelium cultured from patients carrying at least one copy of intronic mutation, showed a correction of the splicing events after AONs incubation compared to control. When possible, chloride measurements have also been carried out after AONs incubation with or without CFTR modulators.

Conclusion: Our findings demonstrated the efficacy of AON-based strategy to restore normal CFTR mRNA. AON-based strategy is an appropriate manner to offer new therapy purposes for CF patients with intronic mutations.

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Repurposed tRNAs to suppress nonsense mutations associated PTCs in Cystic Fibrosis

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Nonsense mutations convert sense codon to a pre-mature termination codon (PTC), which triggers an abrupt termination of protein translation. The ribosome arrest at PTCs can also activate mRNA surveillance pathways resulting in the decay of the PTC-carrying mRNA. Multiple small molecule-based therapies failed in clinical trials as they are not selective to the PTC and cause pervasive readthrough at natural termination codons (UAA, UGA and UAG). We developed a novel strategy to repurpose sense-codon decoding tRNAs into efficient PTC suppressors, that outcompete NMD and restore protein expression in disease-related background. The cystic fibrosis transmembrane conductance regulator (CFTR) has multiple nonsense mutations associated with severe cystic fibrosis phenotype. Using various CFTR mutations we tested the suppressor tRNA efficacy in different cell culture models and patient-derived primary material. The suppressor tRNAs reestablished the CFTR expression and function. In addition, mouse models prove the high safety of our suppressor tRNAs; ribosome profiling demonstrates that the suppressor tRNAs have no off-target effects at natural stop codons in vivo.

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Harnessing CRISPR-Cas9 technology to revert F508del-CFTR defect

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F508del is the most common mutation that cause cystic fibrosis and consists in a 3 nucleotides deletion leading to the loss of phenylalanine 508. Recently, new technologies based on CRISPR have been discovered and tested to correct genetic mutations, offering unprecedent opportunity for the treatment of genetic diseases. Despite that, mutations like F508del, which consist of small deletions, remain difficult to correct. It has been observed that secondary mutations *in cis* with F508del may correct the folding of the channel and partially restore its activity. Therefore, this revertant mutations can be introduced into the F508del locus to counteract the genetic defect as a therapeutic strategy for CF.

We decided to identify novel revertant mutations able to rescue F508del CFTR localization and function. We generated a library of F508del-CFTR mutants introducing random mutations into the F508del-CFTR gene. Cells that presented restored CFTR presence at the plasma membrane (PM) were isolated through flow cytometry analysis and mutations were identified by next generation sequencing. The isolated revertant mutations were evaluated for their ability to restore CFTR maturation and function with flow cytometry, western blot analysis and YFP assay, reaching levels similar to the wild type CFTR.

In addition, we exploited CRISPR base editors to introduce some revertant mutations, such as I539T, R553Q, G550E, R555K and R1070W, in CF cell models. Base editor consists in a Cas9 nickase fused to a cytosine or adenine deaminase enzymes that allow the editing of specific nucleotides without generating DNA double strand breaks. Editing efficiency of several sgRNAs was evaluated through sanger sequencing analysis showing up to 60% of base conversion. PM localization and functionality of the CFTR channel showed different degree of recovery compare to the wild type CFTR.

Overall, we demonstrated that revertant mutations can be used as a potential novel strategy to repair F508del defect through genome editing leading to a permanent correction of the endogenous F508del CFTR gene.

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Low molecular weight alginate oligosaccharides enhance the diffusion of lipid-nanoparticles through cystic fibrosis mucus

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The airway mucus barrier is a major barrier for lipid nanoparticle (LPN) based therapies targeting the human lung epithelium. Mucociliary clearance removes foreign agents from the lung while chronic airway inflammation, often observed in cystic fibrosis (CF), increases the thickness and the viscosity of mucus barrier, which leads very low drug delivery efficiency.

We are developing novel receptor-targeted nanocomplexes (RTNs) for delivery of *in vitro* transcribed mRNA (IVT mRNA) or siRNA to lung epithelial cells as a therapy for cystic fibrosis. The RTN formulation comprises a mixture of cationic lipids and epithelial receptor-targeting peptides yielding cationic nanoparticles. While effective transfection agents, cationic RTNs have poor mucus diffusion properties which could limit their in vivo transfectiojn properties. To increase their mucus penetration efficiency, we combined RTNs with negatively charged low molecular weight alginate oligosaccharides OligoG and OligoM (Algipharma), (Mn 3200 g/mol), anionic and linear structures composed of (1-4) linked β-D-mannuronic acid (M) and its C-5 epimer α-L-guluronic acid (G). Their effects on RTNs were assessed in a mucus diffusion assay using a mucus barrier model comprising CF mucus on polyester membrane transwell inserts with 3.0 µm pores and 50 mM Tris buffer pH 8.0 was filled in the basolateral side in 24-transwell plates. OligoG and OligoM were added directly to the mucus layer before RTNs were added or were formulated with the RTNs.

We first investigated the effects of charge on the mucus penetration efficiency of RTNs containing IVT mRNA, non-PEGylated cationic RTNs (DOTMA/DOPE) and ICAM-1 receptor targeting peptide E displayed very low mucus diffusion rate. The effects of OligoG and OligoM on RTN diffusion were investigated by adding the reagents to mucus, or by formulating with the RTNs themselves. The RTNs prepared by incorporation of OligoG or OligoM into the cationic RTN were found to have anionic surface charge (zeta potential) while the RTNs containing siRNA with OligoM were smaller than those without. OligoG added to cationic mRNA RTNs dramatically improved their diffusion in mucus due to their anionic charge and smaller size. The alginates added to mucus before addition of cationic RTNs also improved the diffusion rate but not as much as those formulated in RTNs. RTNs containing siRNA instead of mRNA, both approaches of adding OligoG and OligoM to mucus or incorporating into cationic RTNs improved the diffusion rate. The alginates added to mucus improved the diffusion rate of the RTNs whilst the reagents formulated in the RTNs did not. However, the improvement was not as high as those in the mRNA RTNs.

In conclusion, OligoG and OligoM promote mucus penetration of cationic RTNs containing siRNA and mRNA, with particlualr benfit for those containing mRNA. These alginates appear to reduce the interaction of RTNs with mucins, and increase fluidity of the mucus itself. Especially OligoG formulated in cationic mRNA RTN significantly increase the penetration efficiency and may enhance the transfection efficiency in CF lungs.

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Transduction of ferret airway, liver and pancreas following a single dose of AAV1

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The major hurdle with gene therapy is the transduction of sufficient CFTR to be therapeutic without adverse events. One way to show definitively that gene therapy can be therapeutic is to rescue defective CFTR function in a relevant animal model that recapitulates, as closely as possible, human disease. The ferret model has undergone extensive development into a highly useful disease model in animals bearing mutations in CFTR such as G551D and F508-del. Demonstration that a gene therapeutic approach can ameliorate disease in these ferrets would be an advance. Thus, the goal here is to assess whether dosing of AAV1-CFTR vectors administered to young ferrets leads to widespread gene transfer and expression. Although an excellent model for studying CF pathophysiology, a recent study found that an inhibitory substance is present in the airway which may alter the transduction of ferret airways by AA1. To test whether we can overcome this potential barrier, we sprayed into the airways of male and female 5-week-old ferrets 1 dose of 10^{11} vg of AAV1-GFP, one male and one female was kept as controls. AAV1-GFP was detectable at $\geq 10^{6}$ vg/ug genomic DNA in trachea, lung, liver and pancreas. mRNA expression was detected at $\geq 10^{5}$ copies/ug genomic DNA in trachea, lung, and liver and 10^{4} copies/ug genomic DNA in pancreas. Widespread immunostaining and protein expression were seen in the tissues of both animals. GFP protein expression following a single dose of AAV1-GFP suggests that transduction by AAV1 based vectors occurs in airways, liver and pancreas of normal ferrets. Given that CF is a multiorgan disease, transduction of liver and pancreas and perhaps the GI tract following lung delivery may be an added benefit for multiorgan rescue of CFTR.

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Optimization and characterization of receptor-targeted nanoparticles (RTNs) for delivery of CRISPR/Cas9 ribonucleoprotein complexes to human airway epithelial cells

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Lipid-based nanoparticles (LNPs) are efficient tools for cellular transfection with nucleic acids and proteins. This can be used to accomplish various goals: delivery of fluorescent reporter proteins, labeled nucleic acids, DNA editing machinery, etc. Furthermore, conjugation of LNPs with receptor-targeting peptides can form receptor-targeting nanoparticles (RTNs), which exhibit specificity to transfect desired cell types. Optimizing the formulation of these RTNs to carry specific cargo is essential. Biophysical properties such as size, homogeneity, and charge can have significant effects on transfection efficiency. Generally, smaller and more homogenous populations are desirable. Cationic charge is also generally desirable for favorable interactions with both negatively charged cargo (nucleic acids and many proteins) and components of cell membranes. However, for some physiological environments, anionic charge can actually be favorable; additionally, inclusion of ionizable “helper lipids” can aid in endosomal escape once the RTN has entered the cell. Our lab has worked to formulate RTNs that efficiently target human airway epithelial cells in order to transfect DNA, mRNA, and CRISPR ribonucleoprotein (RNP).

Four cationic lipids (C14DOPE, C16DOPE, C18DOPE, and the commercially available Lipofectamine) and two targeting peptides (Peptides E and Y, from a phage display study) were investigated for transfection efficiency. Experiments were performed on normal human bronchial epithelial cells (NHBEs) derived from primary cells with DNA, mRNA, and/or CRISPR ribonucleoprotein (RNP), and the combination of C16DOPE and Peptide Y was found to be most effective for transfecting cells of interest. Ratios of targeting peptide were found to significantly impact RTNs’ ability to transfect the human bronchial epithelial cell line 16HBE14o-, with increased transfection efficiency achieved by increasing amounts of peptide. Morphology of RTNs were then imaged via cryo-transmission electron microscopy (Cryo-TEM), and assembly of the RTN in layers (RNP residing between lipid bilayer, and peptide conjugated to outer lipid layer) was observed.

When delivering RNP, mixing order of different RTN components was found to impact the size and homogeneity of RTNs across multiple timepoints. First mixing RNP with lipid, and finally mixing in peptide, yielded the smallest and most homogenous populations, as well as the most consistency among technical replicates when measuring these characteristics. It was also investigated whether the observed level of difference in size and homogeneity yielded significant differences in transfection ability.

Optimized RTNs were found to effectively shield RNP cargo from a fluorescent nucleic acid stain, and the role of RTN components was investigated via this “quenching” ability. Ratios of targeting peptides were not found to significantly affect packaging efficiency, suggesting that its role in enhancing transfection is not encapsulation of cargo, and is most likely solely due to cell targeting.

Eventually, our group hopes to use nebulized RTNs to deliver DNA-editing machinery or *CFTR1* mRNA to the cystic fibrosis (CF) airway *in vivo*. This will be impacted by the biophysical properties investigated above, and our future work aims to characterize RTNs' ability to transfect CF mutant cells in culture conditions that replicate the CF airway.

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Modulation of CFTR intron 22 alternative polyadenylation (ApA) usage may have therapeutic potential for the treatment of certain 3' CFTR PTC variants

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A focus of the CFFT Lab is discovery of agents that promote readthrough for premature termination codons (PTCs). Previously, we showed that the pathogenic CFTR W1282X PTC variant leads to increased usage of intron 22 alternative polyadenylation (APA) sites, resulting in expression of CFTR exon 22 (e22) 3' truncated mRNA that escapes Nonsense-Mediated mRNA Decay (NMD) and produces a CFTR protein with partial function. Furthermore, we showed that intron 22 ApA usage and exon 22/23 splicing are competing events and we hypothesized that blockade of exon 22/23 splicing, would produce a more stable mRNA that translates into truncated CFTR protein with residual function, and may be beneficial for people with CF harboring PTCs downstream of exon 22 (i.e., W1282X). We created a gene edited cell line (16HBEge CFTR W1282X-ΔE23-27) by deletion of exons 23-3'UTR that forces expression of CFTR e22 3' truncated mRNA. This deletion increased expression of CFTR e22 truncated mRNA ~12-fold (n=3) compared to the parental line, produced truncated Band B and Band C CFTR protein by Western blot (n=3), and the cells treated with correctors (VX-661/445) and potentiator (VX-770) generated forskolin-induced Cl^- current that was 16.5%-24.6% (n=3) of the current from CFTR WT-expressing 16HBE14o- cells.

We extended our analysis of the therapeutic utility of blockade of exon 22/23 splicing to promote CFTR e22 3' truncated mRNA expression by testing a series of steric blocking antisense oligonucleotides (ASOs; 22mers) that targeted the exon 22 splice donor (SD) and the exon 23 splice acceptor (SA) sites. The most effective SD and SA ASOs resulted in ~4.3-fold (SD-18), ~3.0-fold (SA-08) and ~7.8-fold (SD-18 & SA-08 combination) increased expression of CFTR exon 22 truncated mRNA compared to untreated WT 16HBE14o- cells. Western blot analysis of 16HBEge W1282X cells treated with SD-18/SA-08 ASOs revealed protein bands (deglycosylated) consistent with the expression of CFTR 1281-truncated protein as well as and CFTR 1239+9AA-truncated protein (exon 22 truncation).

The functional consequence of SD-18 & SA-08 combination treatment in 16HBEge CFTR W1282X cells was assessed in a TECC-24 assay. The cells treated with SA-18 & SA-08 (10 μM/2 μM) and VX-445/VX-661 (3 μM/3 μM) for 48 hours yielded forskolin-stimulated Cl- current of ~13% of the parental CFTR WT-expressing 16HBE14o- cells.

Experiments to assess the effects of SD-18/SA-08 ASOs in primary human cells are underway. These data further support the concept that the blockade of e22/23 splicing to promote expression of CFTR e22 3' truncated mRNA may have therapeutic potential for PTC mutations downstream of exon 22.

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CFTR functional rescue after delivery of nebulized LNP/mRNA to primary human bronchial epithelial cells derived from patients with different CFTR genotypes

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Cystic fibrosis (CF) is an inherited disease caused by mutations in the *CFTR* gene that encodes a chloride channel, CFTR, located on the apical plasma membrane of specialized epithelial cells. CF causes grave damage to the lungs, digestive system, and other organs, with defective pulmonary mucociliary clearance (MCC) being one of the main causes of morbidity and mortality in CF patients. The approval of small-molecule CFTR modulators such as Trikafta significantly improved the quality of life for most CF patients with access to these drugs. However, a significant fraction of the CF patient population is not amenable to the currently approved CFTR modulators. Therefore, the search for universally applicable therapies that promote CFTR function and MCC remains a goal. To address this challenge, ReCode Therapeutics is advancing an mRNA-based treatment to restore CFTR function using optimized CFTR mRNA encapsulated in proprietary lipid nanoparticles (SORT^{TM} LNPs) delivered as an inhaled aerosol directly to the target epithelial cells of the conducting airways.

Initially, we identified a CFTR mRNA sequence and composition with improved stability that is more efficiently translated into functional CFTR protein when compared to the wildtype sequence**.** Subsequently, we formulated the optimized CFTR mRNA with different SORT^{TM} LNPs. CF patient-derived, differentiated human bronchial epithelial (hBE) cells grown at an air-liquid interface were then treated with aerosolized LNP-formulated mRNA using a commercially available mesh nebulizer. Forskolin-induced Cl^- currents measured by transepithelial current clamp (TECC24) recording showed significant CFTR functional rescue. Optimization of the lead SORT^{TM} LNP candidates led to further increases in the Cl^- current observed. Analysis of protein expression by western blot (WB) and immunofluorescence (IF) confirmed the successful delivery of mRNA and expression of CFTR protein, including translation in pulmonary ionocytes and secretory cells. Interestingly, when we investigated six different CF F508del/F508del donors the level of CFTR functional rescue varied. Two main groups were observed, one with significant CFTR functional improvement and a second group with a minimal increase in function. The latter was subjected to more extensive washing of accumulated mucus on the day prior to nebulization which increased the Cl^- flux observed, suggesting an effect of the mucus layer on transfection efficiency. We are currently investigating the ability of the LNP/CFTR mRNA to efficiently penetrate the mucus/ periciliary layer/glycocalyx (PCL-G) in CF patient-derived hBEs.

Our results demonstrate the capability of the ReCode SORT^{TM} LNPs to deliver LNP-formulated CFTR mRNA as an aerosol and increase CFTR function in well-differentiated CF hBE cultures. These preclinical data support further investigation and provide a practical approach to address a significant fraction of the patient population that does not benefit from current CFTR modulator therapy.

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Identification of pharmacological modulators of the calcium-signaling cascade

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Background: Pharmacological modulation of epithelial ion transport mediated by alternative ion channels could bypass the CFTR defect in cystic fibrosis (CF). For this purpose, we considered the stimulation of calcium-dependent chloride secretion mediated by TMEM16A (ANO1) protein as a possible strategy.

Aims: To identify pharmacological activators/potentiators of calcium-dependent chloride secretion in airway epithelial cells.

Methods: Using FRT cells expressing TMEM16A, we screened a maximally diverse small-molecule chemical library (11,300 compounds) with a functional assay based on the halide-sensitive yellow fluorescent protein (HS-YFP). Primary active compounds were tested with a panel of secondary assays to understand the mechanism of action. Such assays included evaluation of intracellular Ca^{2+} mobilization triggered by extracellular agonists or by caged-IP_3 photolysis, analysis of PIP_2 breakdown by phospholipase C, and ion channel recordings on intracellular membranes.

Results: Three compounds were identified to consistently potentiate TMEM16A activity following a purinergic stimulus. Analysis of the mechanism of action revealed that these compounds do not directly act on TMEM16A but modulate calcium signaling at different levels. One compound appears as a potentiator of the purinergic P2RY2 receptor. The second compound is instead a selective potentiator of the inositol triphosphate receptor type 1 (ITPR1). The third compound is a possible modulator of phospholipase C. All compounds were effective in enhancing calcium-dependent chloride secretion in airway epithelial cells from CF patients (nearly 2.5fold increase with respect to control). Moreover, the first and third compound showed an inhibitory effect on ENaC current that may result from enhanced PIP_2 breakdown.

Conclusions: Compounds identified in our study will be useful as tools of research to investigate the regulatory mechanisms that control ion transport in airway epithelia. They can also be considered for the development of therapeutic agents to improve mucociliary clearance in CF.

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SLC26A9 and ATP12A as potential therapeutic targets for cystic fibrosis

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In cystic fibrosis (CF), loss of function of CFTR in the airways is the cause of multiple defects, including dehydration and acidification of airway surface liquid (ASL), and mucus accumulation. These defects impair mucociliary transport and favor the chronic infection and inflammation of the lung (Shteinberg et al. Lancet 2021, 397:2195-2211). Correction of CF abnormalities may be obtained by restoring the function of CFTR with mutation-specific treatments or through airway physiology-directed therapies. Beneficial effects could be obtained by modulating other channels and transporters in order to stimulate CFTR-independent anion secretion (e.g., SLC26A9 chloride channel) or inhibit ASL acidification (ATP12A proton pump) (Mall et al. J Cyst Fibros 2015, 14:561-570). Despite the increasing interest in these targets, their location in the airways and contribution to epithelial homeostasis is poorly known, and specific modulators are still lacking.

In this study, we aimed to define the expression in the airways of SLC26A9 and ATP12A and to generate *in-vitro* models suitable for drug development.

We started with the investigation of SLC26A9 and ATP12A expression in the airways of CF patients and healthy individuals. Epithelial cells were collected by nasal brushing procedure, freshly fixed, and subjected to immunofluorescence analysis. Preliminary assays were established to select the best antibodies and conditions for targets detection. ATP12A was detected in the apical membrane of mucus secretory cells and was found increased in CF airways. Instead, SLC26A9 was poorly to not detected. These findings were confirmed in cultured nasal epithelia, showing increased ATP12A expression under inflammatory conditions, and very low expression of SLC26A9.

Since recent data showed SLC26A9 presence in human airways (Ousingsawat et al. Int J Mol Sci 2022 Mar 10;23(6):2998; Gong et al. NPJ Genom Med 2022 Apr 8;7(1):28), we further investigated its expression in tissue microarrays carrying sections from different portions of the respiratory system. Spatial profiling by RNAscope showed that SLC26A9 is absent or very low expressed (1-2 dots/cell) in most epithelial and glandular cells of the airways, except for rare (<1%) bronchial epithelial cells showing high expression. We identified these cells as pulmonary neuroendocrine cells (PNECs) open type.

ATP12A is one of the main drivers of the ASL acidification found in CF airways and its inhibition can be considered a potential therapeutic approach for CF. To start the search for drug candidates we developed *in-vitro* models with heterologous (Hela cells) or endogenous (BCi-NS1.1 polarized on plastic) expression of ATP12A and designed an antisense-drug approach aimed at ATP12A suppression. A preliminary screening of an antisense oligonucleotides (ASO) library, targeting ATP12A sequence, was completed in the Hela model and resulted in more than 10 highly effective ASOs, which will be validated in the second model.

In conclusion, the finding of PNEC-restricted SLC26A9 expression highlights the need for further study on the physiopathological role of this target and cells in the airways. Importantly, we found antisense-drug candidates able to suppress ATP12A expression, a potential approach for normalizing ASL pH in CF airways.

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BK modulation as a therapeutic target for cystic fibrosis

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Background: Cystic fibrosis is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR), an anion channel present on the apical membrane of airway epithelia. Loss of CFTR function reduces anion secretion into the airway surface liquid (ASL), causing ASL dehydration and an impairment of the innate immune response. CFTR modulator drugs seek to increase the number of functional channels at the cell membrane and the CFTR open probability. However, the apical membrane potential sits close to the reversal potential for chloride. Thus, physiologically, it seems that the co-activation of potassium channels is necessary to provide the driving force for anion secretion. One such channel is the large conductance calcium-activated potassium channel (BK channel) [1] that also resides on the apical membrane of human bronchial epithelium.

Aim: To investigate the role of BK channels both theoretically and experimentally.

Theory: We first examined BK channel modulation by extending a model of ion transport in epithelial cells first developed by O'Donoghue et al. [2]. The extended model included an ASL compartment where depth and ion concentrations could be predicted.

Experiment: We next tested BK channel activation experimentally using CF-donor cells carrying R334W/ΔF508 CFTR mutation. Cells were grown in PneumaCult™-Ex Plus and PneumaCult™-ALI Medium to create air-liquid interface (ALI) cultures in the absence of antibiotics and antimycotic agents. We used BK channel activator GoSlo-SR-5-6 [4] (GoSlo) to modulate the BK channel. GoSlo lowers the voltage required for half maximal activation for all BK channels, independent of their subunit composition, by about 50 mV, allowing them to stay open at relatively hyperpolarised potentials. To measure ASL depth we used the method of Ivanova et al. [3].

We investigated the effect on ASL depth on HBE cultures by measuring a control with DMSO (vehicle) applied to the basolateral side of the culture vs GoSlo (10 uM), also applied basolaterally or GoSlo applied first vs DMSO only 24 hrs later.

Results and conclusion: A simple theoretical model of ion transport in airway epithelia predicts that activation of basolateral BK channels will increase airway hydration, in agreement with data published by Manzanares and colleagues [1]. This prediction is well-matched by the experiment which shows an increase in ASL depth from the DMSO baseline.

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Identification of predictive biomarkers from the graft associated with the development of primary graft dysfunction after lung transplantation in CF patients

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Background: Lung transplantation is the only therapeutic option for patients with a terminal lung condition associated with cystic fibrosis (CF). Unfortunately, survival rates at 5-yrs after lung transplantation (65%) remain too low. Primary graft dysfunction (PGD) is the first cause of death in the perioperative period and is associated with the development of acute respiratory distress syndrome, higher risks of lung infection, chronic rejection, and lower survival rates. We hypothesized that alveolar epithelial dysfunction in the donor graft, after cold ischemia and inflammation, is a critical component of PGD pathophysiology in lung recipients.

Objective: The goal of this study was 1) to study the impact of inflammation and ischemia on alveolar functional integrity and 2) to identify markers of alveolar damage and dysfunction within the donor grafts and subsequent development of PGD in lung recipients.

Methods: We first used a model of alveolar primary cultures, submitted to a protocol mimicking cold ischemia in a preservation solution, and then porcine models of ischemia/ex-vivo reperfusion and induced inflammation. We also collected the clinical data from 172 lung donors and their recipients (including from 67 CF patients) as well as blood, bronchoalveolar lavages (BAL), and lung tissues from the donors and the recipients at different time points, i.e. before, during and after lung transplantation (in the perioperative period at the ICU and follow-up visits at the CHUM transplantation clinic). The PGD grade was determined according to guidelines from the International Society for Heart and Lung Transplantation. The levels of inflammatory, alveolar damage/integrity, and functionality markers were then determined.

Results: In primary alveolar cell cultures, we showed reduced tight junction (ZO-1) protein, ENaC and CFTR expression as well as a decline in transepithelial resistance and wound healing rates following the cold ischemia protocol. In the porcine models, we observed lung damage and edema flooding, with exacerbated inflammation response, associated with a decrease in ZO-1, ENaC and CFTR expression, after ischemia/reperfusion injury. Among the 172 lung transplants we recruited at the CHUM transplantation center between 2013 and 2020, 39% were CF patients. 26% of recipients developed a PGD (grade 2/3) in the perioperative period. We found that the duration of cold ischemia of the graft, although below the recommended threshold, is associated with increased PGD scores. Histological analysis of lung biopsies from the donor graft before the transplantation showed alveolar inflammatory infiltration and epithelial damage. Immunostainings of lung sections and alveolar epithelial cells isolated from the donor grafts revealed reduced expressions of integrity (ZO-1) and functionality (ENaC, CFTR) markers. Importantly, we observed a relationship between reduced ENaC/CFTR channel expression, higher cold ischemia duration, and PGD scores.

Conclusion: This study provides novel insights into epithelial damage/dysfunction within the donor graft associated with PGD development in the recipient and paves the way for the identification of novel predictive biomarkers and therapeutic targets. Our ultimate goal is to develop strategies for the restoration of alveolar integrity and functionality of the graft and thus to prevent the development of PGD in lung transplants.

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P81

Identification of novel NMD modulators to rescue nonsense mutations in cystic fibrosis

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Background: The rescue of CFTR in cystic fibrosis (CF) patients with premature termination codons (PTCs) is an urgent unmet need. The nonsense-mediated RNA decay (NMD) is a phenomenon that limits the rescue of CFTR when PTCs are present. We recently demonstrated that the NMD inhibitor SMG1i strongly increases the mRNA of CFTR with a variety of PTCs (Y122X, G542X, R553X, R1162X), but this effect does not result in CFTR rescue, probably because inhibition of SMG1 also impairs PTC readthrough. In contrast, W1282X, which does not strictly require readthrough, can be rescued with SMG1i plus correctors.

Aims: Our aim is to find novel NMD inhibitors acting with a mechanism different from that of SMG1i. For this purpose, we are screening a chemical library with a CFTR functional assay.

Methods: The 16HBE14o- cell line with the W1282X mutation (obtained from CFF) was stably transduced with an expression vector coding for the halide-sensitive yellow fluorescent protein (HS-YFP). These cells are used to screen a chemical library containing more than 9,000 approved drugs, investigational drugs, and compounds with known biological activity. The most active compounds will be evaluated in short-circuit current recordings on 16HBE14o- cells with different PTCs. Most promising molecules will be tested in airway epithelial cells derived from CF patients with PTCs.

Results: We have defined the optimal conditions to screen the library. Cells are treated for 24 h with test compounds in combination with VX-809 and VX-445. Negative and positive controls correspond to cells treated with vehicle alone or SMG1i (plus correctors). After treatment, CFTR function is evaluated with the HS-YFP assay. We have currently screened ~3,000 compounds and different positive hits have been identified. Among these, we found a molecule (NMDi-01) that induces a 4-fold increase in CFTR activity compared to negative control, an effect larger than that of SMG1i. We tested NMDi-01 at multiple concentrations to generate a dose-response relationship and to assess its potency (EC_{50} = 0.65 μM). We are carrying out additional experiments to establish the MOA. Interestingly, NMDi-01 is ineffective on F508del-CFTR thus suggesting that it acts as a real NMD inhibitor and not as a general CFTR gene transcription enhancer/amplifier. To confirm this conclusion, we will quantify, by real time RT-PCR, changes in CFTR mRNA levels in cells with/without PTCs. In parallel, we are continuing the screening of the library and the validation of other primary hits.

Conclusions: We expect to discover NMD inhibitors as well as novel biological targets and processes associated with NMD.

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TAVT-135, a novel chloride ion transporter for the pan-genotypic treatment of cystic fibrosis: electrophysiological and mucus-hydration properties

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Objectives: The treatment of cystic fibrosis (CF) has been transformed by the introduction of modulators of the CF transmembrane conductance regulator (CFTR). However, approximately 10% of patients with CF have ineligible genotypes. TAVT-135, a novel small molecule-peptide conjugate composed of a chloride ion-binding moiety and a cell-penetrating peptide (CPP), is being investigated as a potential treatment for CF, regardless of CFTR mutational status. Here, we report the results of a series of studies that were performed to characterize the electrophysiological and mucus-hydration properties of TAVT-135.

Methods: The effect of TAVT-135 (10 μM) or its separate functional components on intra- to extracellular chloride ion transport was evaluated in *Xenopus laevis* oocytes using a two-electrode voltage-clamp technique.

Anion efflux was then evaluated by means of a modified Ussing chamber system in human bronchial epithelial (HBE) cells harboring mutations for non-functional CFTR. TAVT-135 (0.01, 0.1, 1, 10, 25, and 50 μM) was acutely added to the apical side of the cultures. Short circuit current (I_{SC}) following amiloride-induced inhibition of the epithelial sodium channel (ENaC) was measured, along with transepithelial electrical resistance (TEER).

Mucus hydration in HBE cells with CFTR mutations was assessed by measuring the height of the airway surface liquid (ASL) and the periciliary layer (PCL). Following chronic exposure to TAVT-135 (1, 10 and 100 μM) for 48 hours, ASL and PCL were visualized with apical application of FITC-dextran; z-stack images were obtained by confocal microscopy to determine heights (μm).

Results: In *X. laevis* oocytes, TAVT-135 induced a rapid chloride efflux, demonstrating chloride transport from the intracellular to the extracellular space. The CPP alone resulted in a slight change in chloride current, but the magnitude of response was typically around 5% of TAVT-135. The unconjugated chloride-binding component did not have any detectable effect on chloride current.

In HBE cells, there was a statistically significant, dose-dependent increase in I_{SC} following the acute application of TAVT-135 at concentrations ≥1μM, demonstrating anion efflux. Following 5 minutes of exposure, TEER was maintained at TAVT-135 concentrations ≤1μM and decreased at concentrations ≥10μM.

TAVT-135 (10 and 100 μM) statistically significantly increased ASL and PCL height in HBE cells with CFTR mutations after 48 hours of incubation compared with the untreated control.

Conclusions: In these *in vitro* experiments, TAVT-135 rapidly induced intracellular chloride transport across plasma membranes without negatively impacting the epithelial barrier. TAVT-135 also promoted ASL hydration and increased PCL height, which may suggest a mucociliary clearance effect *in vivo*. These data support that TAVT-135 has the potential to address significant unmet needs in patients with CF, including those who are ineligible for, or not responding to, CFTR modulators.

P83

Gene-targeting approach to investigate the role of TMEM16A (ANO1) in airway epithelia

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Background: TMEM16A (ANO1) is a plasma membrane protein that works as a calcium-activated chloride channel. TMEM16A could be an important alternative target for patients affected by cystic fibrosis (CF) in which there is a defective activity of the CFTR chloride channel. As already published by our group, TMEM16A expression and function is up-regulated under inflammatory conditions (Scudieri et al., J Physiol 2012), particularly those associated with mucus hypersecretion (e.g. IL-4 treatment). The role of TMEM16A in supporting mucus secretion and mucociliary transport is unclear. Also, TMEM16A may have an additional role in the modulation of calcium signaling.

Aim: To analyze the role of the TMEM16A channel in calcium signaling and its contribution to the air surface liquid (ASL) properties using a knockdown approach in differentiated bronchial epithelial cells.

Results: We nucleofected basal stem cells with Cas-9 protein and a mix of three gRNAs targeting three different TMEM16A exons (16A-KD treatment). As control, we nucleofected Cas-9 without gRNAs. After nucleofection, cells were plated on Transwell inserts and allowed to differentiate under air-liquid interface (ALI) condition. After two weeks, epithelia were treated for 72 h with/without IL-4 and then studied by short-circuit current recordings. In IL-4 treated epithelia, the peak of current elicited by UTP 10 μM decreased from 92.1 ± 24.0 to 7.2 ± 1.2 μA/cm^2 in 16A-KD epithelia. Analysis of epithelial lysates by immunoblot, confirmed a strong decrease in TMEM16A protein expression in 16A-KD epithelia. To analyze calcium signaling, we used the Fluo-4 fluorescent probe. Stimulation with UTP (10 μM) evoked a similar calcium signal irrespective of TMEM16A knockdown.

Conclusions: As expected, TMEM16A expression knockdown markedly decreases calcium-dependent chloride secretion. In contrast, the calcium mobilization elicited by a purinergic stimulus is unaffected by TMEM16A downregulation. Analysis of ASL properties, particularly viscosity, in 16A-KD epithelia is in progress. In general, the method based on nucleofection of basal stem cells with Cas9/gRNA complexes, followed by generation of differentiated epithelia without selection of targeted cells seems very effective, at least for TMEM16A. This method could be similarly useful for other epithelial targets.

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Targeting the oxidative imbalance for CFTR rescue in cystic fibrosis

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Background: Cystic fibrosis (CF) is a life-shortening genetic disorder, caused by mutations in the CFTR gene. CFTR mutations are divided according to their implication in the channel proteostasis (classes I-VI). Among all, F508del (class II) is the globally common CFTR variant responsible for the premature degradation of the misfolded protein [1].

Inefficient maturation and accumulation of F508delCFTR fragments by the proteasome system lead to ER stress, persistent inflammation and oxidative imbalance caused by an overproduction of ROS. Specifically, high ROS and inflammation in CF are associated with a marked depletion of glutathione (GSH) which fosters the oxidative damage of polyunsaturated fatty acids (PUFA) via lipid peroxidation (LPO). Similarly, low levels of NF-E2-related factor 2 (Nrf2), regulator of GSH synthesis, were observed in CF cell lines [2].

The current therapy (Trikafta®) is addressed to an extremely limited number of mutations including F508del, consequently the search for new therapeutic proposals is still urgent.

Objectives: The project points to re-equilibrate the redox homeostasis by testing Nrf2 activators to increase GSH levels and LPO inhibitors, supposing that restoring the derailed CF intracellular environment might improve mutated CFTR proteostasis and its functional recovery.

Methods: CFBE41o- parental or stably transfected with WT- or F508del-CFTR were used as CF cell models. Upon 24h-treatment with Nrf2 activators, transcription factor level and activity were assayed by WB and transfection with Nrf2 reporter plasmid, respectively. Moreover, GSH quantitative determination was assessed with Tietze assay. In parallel, ROS and LPO levels were detected using fluorescent probes (H_2DCFDA, C11-Bodipy respectively) after 24h-treatment with lipid peroxides scavengers. CFTR trafficking at the plasma membrane (PM) was followed by WB. Then, the most promising compounds have been tested in Ussing Chamber to evaluate channel function. To this, WT- and F508delCFTR fisher rat thyroid (FRT) cells were also employed.

Results: The project demonstrates that acting on the redox imbalance in CF models can recover F508delCFTR at the PM.

Nrf2 activators tested on CF cell lines rescued the transcription factor levels and activity. In fact, the reporter signal was stronger in cells transfected with the Nrf2 reporter plasmid and treated with the selected activators. Moreover, these compounds increase total glutathione and, specifically, the reduced fraction. Interestingly, treatments rescue the core-glycosylated CFTR and this paves the way for a combination with correctors.

High ROS and LPO levels were detected in cells expressing defective CFTR. Indeed, a decrease in LPO induced by specific radical-trapping molecules was noticed through C11-Bodipy technique. These molecules, in specific combinations with correctors, increase the channel trafficking at the PM in cell models and restore CFTR-mediated ion transport in reproduced CFBE and FRT epithelia providing a greater effect than the corrector alone.

Conclusions: The obtained data suggest new therapeutic alternatives that could implement the therapeutic approach in clinical use. Being not directed against the mutated CFTR, this proposal has the potential to be translated to orphan mutations.

References:

[1] C.M. Farinha et al., FEBS J, (2016);283(2):246-64.

[2] A.G. Ziady et al., Int J Biochem Cell Biol, (2014);52:113-23.

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P86

Characterization and activation of adenosine receptor pathway in CFTR function

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Adenosine receptors (ARs) signalling modulates a variety of physiological processes. ARs have been subclassified in two major subtypes, according to their ability to increase (A_{2A} and A_{2B}) or inhibit (A_1 and A_3) cyclic adenosine monophosphate (cAMP) production. cAMP is involved in the activation of some proteins as cystic fibrosis transmembrane regulator (CFTR). Pathogenic variants of CFTR gene cause the autosomal recessive disease cystic fibrosis (CF). Consequently, pharmacological manipulation of ARs is of potential interest to study CFTR modulation. We aim to characterize the expression of ARs in intestinal organoids from non-CF individuals by RT-qPCR. In addition, we study how the modulation of ARs are involved in CFTR activation using N-ethyl-carboxamide adenosine (NECA) during the forskolin-induced swelling (FIS) assay. NECA is a synthetic adenosine analogue which binds non-selectively to all AR subtypes. Our preliminary results show that A_{2B} subtype is the predominant receptor expressed in intestinal organoids. Similar data is observed employing the functional in vitro assay, which points to a link between ARs signalling and CFTR function. In conclusion, the expression of ARs in intestinal epithelial cells suggests that the modulation of the ARs pathway, especially the A_{2B} subtype, could be investigated to modulate CFTR protein function.

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Aminoglycosides and eRF3a degraders synergize for efficient functional restoration of native CFTR PTC variants

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Drugs in clinical trials for readthrough of cystic fibrosis-causing nonsense mutations have either failed (ataluren) or not yet demonstrated meaningful efficacy (ELX-02). There remains an unmet need to develop effective readthrough therapy for ~10% of people with cystic fibrosis (PwCF), who carry CFTR nonsense mutations, in particular for those who are ineligible for highly effective modulator treatment based on their genotype (2-3% of PwCF). Recent *in vitro* studies [1,2], combining translation termination attenuation with premature termination codon (PTC) readthrough active compounds, such as aminoglycoside G418 or the chemically related clinical candidate, ELX-02, demonstrate synergistic and efficacious restoration of full-length CFTR at levels likely to confer a clinical benefit.

We tested combinations of aminoglycosides (G418 and ELX-02) and degraders of the translation termination factor eRF3a (CC-885 and the clinical development candidate CC-90009 [3]) for the translational readthrough of native CFTR R1162X in a CRISPR engineered 16HBE cell line (CFF-16HBEge CFTR R1162X). The combination treatment achieved full-length CFTR protein levels of 20% of normal (expression in parental 16HBE14o- cells) in CFTR ELISA and Western blot assays with Band C as the dominant form of CFTR. In addition, functional restoration (as measured by TECC-24 equivalent current I_{eq} assay) of CFTR R1162X by ELX-02 was significantly (~3-fold) enhanced in the presence of CC-90009 (1 uM). Synergistic effects of the combination treatment were also detected in isogenic 16HBEge cell lines expressing CFTR Y122X or CFTR G542X and in primary human bronchial epithelial (hBE) cells from two donors with nonsense mutation genotypes ($G542X^{+/+}$ and G542X/R553X).

Building on this concept, we utilized CC-90009 to sensitize a high throughput screening assay to identify novel readthrough modulators that may synergize with an eRF3a degrader mechanism. To date, we screened in the 16HBEge CFTR R1162X cell line 50,000 compounds from four chemically diverse libraries and validated 92 hits, including at least two ribosomal modulators. The next steps will focus on combinational profiling of hits to explore putative synergies for the readthrough of CFTR PTC variants and down-selection of hits for hit-to-lead optimization.

[1] Sharma et al., Nature Communications 2021; 12:4358. 10.1038/s41467-021-24575-x

[2] Baradaran-Heravi et al., Nucleic Acids Research 2021; 49:3692. 10.1093/nar/gkab194

[3] Hansen et al., J. Med. Chem. 2021, 64:1835 10.1021/acs.jmedchem.0c01489

P88

The role of electrogenic and electroneutral monocarboxylate transport in airway clearance

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Introduction: Monocarboxylates can be transported by an electrogenic Na^+-coupled system that corresponds to the SMCTs (SLC5 family) and an electroneutral H^+-coupled system that correspond to MCTs, (SLC16 family). Previously, MCT2 and MCT4 have been described in human bronchial epithelium and localized in apical and basolateral membrane, respectively. But there is no evidence of SMCTs activity in this tissue. In here, we describe for the first time that SMCT1/SCL5A8 is functionally expressed in mouse tracheal epithelium. We hypothesized that the activation of monocarboxylate transport might down regulate airway clearance as it is known that increased Na^+ absorption reduce airway surface liquid (ASL), and increased H^+ secretion acidify ASL. Then we tested if mucociliary clearance is affected by MCT and SMCT activity in mouse airways.

Methods: Short-circuit current in mouse tracheas was measured in Ussing chambers (n=4-5 for each group), expression of transporters by qRT-PCR (n=3), particle track speed (PTS; n=4) and mucus transport speed (MTS; n=4) by videomicroscopy. Localization of MCT2 and MCT4 was evaluated by immunofluorescence. Animals (C57BL6/J) were housed at CECs-Animal facility under controlled temperature and humidity with free access to water and food. All protocols were approved by the IACUC (#CECs-2022-04), in accordance with relevant guidelines and regulations.

Results: 10mM apical L-lactate and D-lactate induced a negative current and was Na^+-dependent. qRT-PCR assay determined that SLC5A8/SMCT1 was highly expressed in airway epithelium compared to SLC5A12/SMCT2. The activation of SMCT1 showed a reduction in amiloride sensitive Na^+-absorptive currents in tissues previously treated with L-lactate compared with non-treated. In addition, L-lactate, which is transported by SMCT and MCT induced an increase in PTS, that was prevented by addition of MCT1/2 inhibitor (AR-C155858) in the mouse tracheas. On the other hand, D-lactate that is exclusively transported by SMCT did not affected PTS. Furthermore, analysis of mucus transport determined that L-lactate but not D-lactate increased the speed of mucus, that was prevented when tissues were incubated with AR-C155858. Surprisingly, immunofluorescence determined that MCT2 and MCT4 are localized in basolateral membrane. Thus, our results indicate that the effect on PTS and mucus transport induced by L-lactate possibly is mediated by MCT1. Preliminary data indicated that in presence of MCT1 inhibitor (AZD3965) this effect on speed of mucus was diminished.

Discussion: Monocarboxylate uptake by MCTs removes H^+ from ASL, alkalizing the airway surface and improving airway clearance. The use of MCTs transportable substrates might help alleviate mucostasis in muco-obstructive diseases and will be tested in animal models of these diseases.

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Effect of Tristetraprolin overexpression in CF

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Objectives: Regulatory elements like microRNA (miRNA) and RNA-Binding protein (RNA-BP) act independently or in synergic/antagonist manner by binding 3'UTR extremity of mRNA, inducing their degradation or inhibition of their translation. Tristetraprolin (TTP) is an anti-inflammatory RNA-BP, reported to be deregulated in CF cultures. TTP, when non-phosphorylated, destabilizes mRNA of pro-inflammatory proteins, like IL-6, IL-8 or TNFα, participating in resolution of inflammation. We aim to increase the expression of TTP proteins to control mechanisms of the inflammatory response.

Materials and methods: Reporter gene vectors containing the 3'UTR regions of TTP, IL-8, IL-6 and TNFα; have been constructed. The luciferase activity has been measured to evaluate the importance of different regulatory elements (miRNAs and RNA-BP). Overexpression of TTP has been generated by CRISPR activation (CRISPRa) assay, by expression vectors use or by an oligonucleotide-based strategy. The effect of TTP on pro-inflammatory cytokines have been evaluated by luciferase activity, mRNA and protein level quantification and ELISA dosage.

Results: In CF cells, we found that TTP mRNA is under-expressed. Overexpression of TTP by CRISPRa or TTP expression vector, induced an increase in TTP mRNA and a reduction of pro-inflammatory cytokines mRNA level, suggesting that TTP mRNA level elevation led to inflammation resolution. We next look for regulatory elements that can regulate TTP expression.

Study of TTP 3'UTR *trans*-acting elements showed that the RNA-BP HuR and HuB have an activating effect on TTP luciferase activity. On the opposite way two miRNAs, miR-138 and miR-155, decreased TTP protein level by acting on its 3'UTR. This inhibitory effect is increased in inflammatory context, induced by LPS treatment. The use of miR-138 and miR-155 inhibitors allowed a restauration of TTP protein level. We next designed chemical modified oligonucleotides (TSB, Target Site Blocker) that prevent miRNAs and RNA-BP recruitment. Introduction of TSB, encapsulated in cell-penetrating peptides, showed an increase in mRNA and protein TTP level in CF cells and macrophages. In inflammatory context, TTP mRNA stabilization also led to a strong decrease in cytokine secretions in CF cells and macrophages.

Conclusion: Identify destabilizing motifs on TTP-3'UTR led to conceive new molecular tools for inflammation resolution.

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Fluorescence-based measurements of CFTR channel activity in primary nasal epithelial cultures recapitulates Ussing chamber measurements

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Background:*In-vitro*, bioelectric measurements of responses to CFTR modulators in Human Nasal Epithelial Cell cultures (HNECs) derived from Cystic Fibrosis (CF) patients harbouring certain mutations including the common mutation, F508del, have been shown to correlate with clinical responses in a number of research laboratories (Pranke et al., Am. J. Respir. Crit 2019; Awatade et al., Front Pharmacol. 2018). Thus, there is growing optimism that patient-specific HNECs will be useful as a tool for predicting clinical outcomes for those individuals harbouring rare CF causing mutations. Commonly, the functional response to CFTR modulator combinations in HNEC cultures is assessed by bioelectric measurements, using the Ussing chamber. Ussing chamber studies measure the functional expression of CFTR as a cyclic adenosine monophosphate (cAMP)-regulated chloride channel in the apical membrane of HNECs and is considered as the "Gold standard" in CFTR functional assays. While this method is highly informative, it is relatively time consuming, requires specialized equipment and expertise. A fluorescence-based, multi-transwell method for assaying regulated apical chloride conductance (FI-ACC), promises to enable theratyping in patient-derived HNECs in any laboratory possessing a fluorescence plate reader. This assay is a medium-high throughput membrane potential assay that can also be used to study the CFTR mediated chloride conductance. This assay was previously published by Saumel et al. (NPJ Genom. Med. 2017) using a small sample size. Now, we validated its ability to recapitulate Ussing measurements in a larger cohort of 41 subjects harboring multiple CFTR genotypes, supporting its future application to the study of alternative chloride channels in primary nasal tissues.

Aim: In the current study we aim to validate the fluorescence-based, medium-high throughput *in-vitro* assay of CFTR chloride channel activity using patient specific HNECs.

Methods: HNECs were obtained from 41 CF patients, homozygous for F508del, G551D or W1282X and 8 non-CF subjects and expanded to passage 3 as described by Laselva et al. (Eur. Respir. J. 2021). These HNECs were maintained under air liquid interface conditions to obtain fully differentiated cultures. Functional studies were carried out on these cultures matching the samples by Ussing chamber under open circuit conditions and FI-ACC measurements for CFTR mediated apical conductance.

Results: Ussing and FI-ACC studies of patient derived cultures reveals genotype specific modulator responses. We validated the use of FI-ACC assay for ranking modulator responses in cultures from people with various CF-causing mutations. Most importantly, we found that there was an excellent correlation ($r=0.71$, $p < 0.0001$) between the apical CFTR channel activity measured by the two assays. Interestingly, FI-ACC shows more sensitivity than the Ussing chamber method for detecting responses to investigational compounds targeting W1282X in nasal cultures derived from donors who are homozygous for the nonsense mutation: W1282X.

Conclusion: The FI-ACC will be a complementary to other assays of bioelectric properties of CF patient derived nasal cultures and it may be potentially useful for the validation of therapeutic strategies targeting alternative chloride channels in these tissues.

P92

An upstream airway-specific enhancer contributes to SLC6A14 modification of lung phenotypes in CF

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Genome wide association studies (GWAS) identified SNPs in the immediate and distant upstream regions of *SLC6A14* associated with meconium ileus (MI) and lung disease severity in individuals with CF, respectively. *SLC6A14* is a sodium- and chloride-dependent neutral and cationic amino acid transporter that is expressed in several organs and co-expressed with CFTR in lung tissue. We previously reported that the associated variants are expression quantitative trait loci (eQTLs) for *SLC6A14,* where alleles associated with reduced MI risk and better lung function show less *SLC6A14* expression in adult pancreatic tissue from the genotype expression project and in naïve CF human nasal epithelia (HNE), respectively. With an expanded resource of >100 CF HNEs we provide additional support that the distant region contains *SLC6A14 eQTLs* (min p=2.69E-05), despite being 206Kb upstream of the transcription start site (TSS). These *airway model* eQTLs colocalize with the lung GWAS SNPs ($p < 0.00001$), indicating that the lung disease association is mediated by SLC6A14 expression. To understand the distinct GWAS signals, the eQTL variants and the mechanisms by which they influence CF phenotypes, gene regulatory bioinformatics was used to prioritize segments followed by reporter gene expression studies to establish promoter and enhancer functions of the associated regions.

Interrogation of the region immediately upstream of *SLC6A14* identified a core promoter of 381 nucleotides and delineation of local haplotypes in pancreas and lung-derived lines pointed to two SNPs within 1.1kb of the TSS as contributing to SLC6A14 expression variation. The distant lung GWAS interval highlighted three sub-regions with potential regulatory function. Of these, the most distal sub-region had support for conformational contacts, direct transcription factor binding and chromatin accessibility in airway cells. Reporter gene testing of this distal sub-region alone displayed no promoter activity but exhibited marked enhancer activity when juxtaposed (>40 fold increase, in either orientation) with the identified core promoter in lung-derived, but not pancreas-derived, cell lines. SNPs within this fragment were then examined for their influence on *SLC6A14* expression. Notably, the C allele of rs4446858 that demonstrated genome-wide significance with greater lung function in the GWAS (p=1.69E-09) and lower *SLC6A14* expression (p=0.000165) in eQTL analysis of HNE, aligns better with the IRF1/2 consensus binding site. Expression vectors with the C allele showed boosted expression compared to the T allele with addition of exogenous IRF1, but not with IRF2 or both IRF1 and IRF2. Involvement of the IRF1/2 innate immunity responders align with observations that lung disease associated variants in this upstream region are also associated with age of chronic infection of *Pseudomonas aeruginosa* (min p=0.036) in patients. Further, increased coordinated levels of SLC6A14 with IRF1 and IRF2 transcripts were observed in CF HNE with p=8.81E-10 and p= 3.53E-05, respectively and in single-cell analysis.

The distinct GWAS association signals of the CF phenotypes are explained by tissue-specific regulation of SLC6A14, where airway tissue engages a potent upstream enhancer element to help drive expression. The inclusion of IRF innate immunity responsive elements suggest that modification of lung function in CF by SLC6A14 may directly or indirectly involve response to infection.

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Antisense oligonucleotide targeting TMEM16A: a mutation agnostic therapy for cystic fibrosis

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Background: Although tremendous progress has been made in the cystic fibrosis (CF) field, CFTR modulators cannot treat people with CF-carrying CFTR nonsense mutations or others that do not respond optimally. Hence, alternative approaches are essential to address those unmet needs. Our group is developing an antisense oligonucleotide (TMEM16a ASO) for treating any patient with CF regardless of their mutations. The ASO targets the alternative chloride channel Anoctamin-1 (TMEM16A), previously shown to be downregulated by microRNA-9 in CF, and increases its expression and activity to compensate for defective CFTR. Here, we evaluate the effectiveness of the TMEM16A ASO in CF primary cells bearing different mutations and compare the results of the ASO to different CFTR modulators.

Methods: Well-differentiated ALI cultures were treated by either TMEM16A ASO or control ASO and then mounted in Ussing chambers. CFTR and TMEM16A-dependent short-circuit currents were recorded upon adding amiloride, followed by forskolin, CFTRinh-172, and UTP. Mucociliary clearance was evaluated by tracking fluorescent beads added apically to differentiated ALI cultures after the treatment by TMEM16A ASO or CFTR modulators.

Results: CF differentiated primary cells treated with TMEM16A ASO show an increase in short-circuit currents when stimulated with UTP after TMEM16a ASO treatment. This increase is similar to non-CF differentiated primary cells treated with TMEM16A ASO. TMEM16A ASO had no effect when the cells were pre-treated with TMEM16A inhibitor (Ani9), indicating its specificity to TMEM16A. We also demonstrated an increase in mucociliary clearance in CF differentiated primary cells treated (F508del/F508del; 1717-1G>A/F508del; 2184delA/W1282X). The increase in cells bearing class II mutations was more significant than in the cells treated with Orkambi. We observed an additive effect when TMEM16A ASO treatment was combined with the CFTR modulator.

Conclusions: Our results demonstrate that this strategy could be applied to all people with CF and support the progression of TMEM16A ASO toward a clinical trial.

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