23rd Scientific Meeting

Cystic Fibrosis Omics: From Complex Alleles to Host–Pathogen Interactions

21st – 22nd May 2026

Bonn (Germany)

MUKOVISZIDOSE
Mukoviszidose Institut gGmbH

Organization

Scientific advisory board of the German Research Community for Cystic Fibrosis (FGM) & Mukoviszidose Institute gGmbH (MI)

Chairs

Laura Schaupp (DE/Berlin), Julia Hentschel (DE/Leipzig),
Burkhard Tümmler (DE/Hannover)

Location

Gustav-Stresemann-Institut
Langer Grabenweg 68
53175 Bonn-Bad Godesberg

Table of content

Please note that not all talks have a published abstract.

Program

Thursday, May 21st

1:00-1:15 pm — **Opening of the meeting**

Chairman of the Forschungsgemeinschaft Mukoviszidose (FGM)
Michael Hogardt (DE/Frankfurt)

Chairs of the meeting
Laura Schaupp (DE/Berlin), Julia Hentschel (DE/Leipzig), Burkhard Tümmler (DE/Hannover)

1:15-2:55 pm — **Session I: Translating CFTR Variant Complexity into Targeted Therapeutic Strategies**
chaired by Julia Hentschel (DE/Leipzig)

1:15-1:45 pm — **Getting the most of CFTR-France to classify variants and complex alleles in the CFTR gene**
Caroline Raynal (FR/Montpellier)

1:45-2:00 pm — **CFTR Complex Alleles in Individuals with Cystic Fibrosis Showing Limited Response to Elexacaftor/Tezacaftor/Ivacaftor (ETI) Triple Modulator Therapy***
Maike Karnstedt (DE/Leipzig)

2:00-2:10 pm — **Effectiveness of ETI in CF patients with complex alleles of the CFTR gene***
Tatjana Jakjovska Maretti (MKD/Skopje)

2:10-2:25 pm — **Targeted Nanopore Sequencing as a Key to Improving CF Diagnostics: Characterization of Splicing Variants to Increase Diagnostic Yield***
Simone Ahting (DE/Leipzig)

2:25-2:40 pm — **Transcriptomic analysis of G542X-CFTR bronchial epithelial cells indicates inhibition of nonsense mediated decay by IL-4 treatment***
Anna Borrelli (IT/Pozzuoli)

2:40-2:55 pm — **Identification of novel pharmacological inhibitors of the nonsense-mediated RNA decay mechanism to rescue CFTR function in patients carrying nonsense mutations***
Arianna Venturini (IT/Pozzuoli)

2:55-3:30 pm — *Break*

3:30-5:00 pm — **Session II: Precision Therapeutics: Functional Profiling and Next-Generation Modulators**
chaired by Burkhard Tümmler (DE/Hannover)

3:30-4:00 pm — **Leveraging Single-Cell RNA Sequencing to Reveal Tissue-Specific Effects of ETI Therapy in Pediatric Cystic Fibrosis**
Saskia Trump (DE/Berlin)

4:00-4:15 pm — **Expanding the therapeutic landscape: Vanzacaftor-Tezacaftor rescues orphaned ETI-resistant genotypes***
Nicoletta Pedemonte (IT/Genoa)

**submitted Abstracts*

4:15-4:30 pm	**Primary nasal epithelial cells for personalized medicine in cystic fibrosis patients with rare mutations*** Jasmin Berger (DE/Berlin)
4:30-4:45 pm	**Multi-omics Analysis of Airway Epithelial Cells following CFTR Modulator Therapy*** Mengjie Ma (CH/Geneva)
4:45-5:00 pm	**Omics to understand the side effects of CFTR Modulators*** Andrea Armirotti (IT/Genoa)
5:00-5:30 pm	*Break*
5:30-6:30 pm	**Keynote-Session** *chaired by Burkhard Tümmler (DE/Hannover)* **Proteostasis landscapes of rare CFTR variants to emerging correctors and off-targets of Elexacaftor/Vanzacaftor** Lars Plate (USA/Nashville)

Friday, May 22nd

9:00-11:00 am	**Session III: Targeting the Airway Interface: Microbial Persistence, Immunity, and Clinical Outcomes** *chaired by Burkhard Tümmler (DE/Hannover) and Laura Schaupp (DE/Berlin)*
9:00-9:30 am	**Integrative computational analysis of longitudinal effects of Elexacaftor/Tezacaftor/Ivacaftor on sputum microbiome and proteome in patients with cystic fibrosis** Laura Schaupp (DE/Berlin)
9:30-10:00 am	**Characterization of mucin glycan structures** Gaël Vos (DE/Berlin)
10:00-10:15 am	**Deficiency of a repressor of maltodextrin metabolism in commensal *Streptococcus* correlates with *Pseudomonas aeruginosa* growth inhibition*** Sébastien Boutin (DE/Lübeck)
10:15-10:30 am	**Characterization of mucoid hyper biofilm forming *Staphylococcus aureus* isolates recovered from the airways of a person with cystic fibrosis with a double mutation in the ica operon*** Christine H. Rumpf (DE/Münster)
10:30-10:45 am	**IL-17A and IL-17F differentially modify transcriptomic profile of human bronchial epithelia*** Daniela Guidone (IT/Naples)
10:45-11:00 am	**Linking highly neutralizing human antibodies targeting multidrug-resistant *Pseudomonas aeruginosa* to clinical and immunological phenotype in people with cystic fibrosis*** Robert Brock (DE/Köln)
11:00-11:30 am	*Break*

**submitted Abstracts*

11:30-12:45 pm **Session IV: Developmental and Epigenomic Network Dynamics in Airway Disease**
chaired by Laura Schaupp (DE/Berlin)

11:30-12:00 pm **Microbial and fungal dynamics in the respiratory tract of infants with cystic fibrosis**
Ruth Steinberg (CH/Bern)

12:00-12:15 pm **The airway microbial metagenome of people with cystic fibrosis and bronchiectasis***
Ilona Rosenboom (DE/Hannover)

12:15-12:45 pm ***Late breaking science talk:***
Restoring near-normal F508del-CFTR function: a cell-permeable nanobody synergizes with CFTR modulators in primary airway epithelial cells from patients with cystic fibrosis
Tihomir Rubil (DE/Berlin)

12:45 pm **Closing of the Meeting**

**submitted Abstracts*

Session I: Translating CFTR Variant Complexity into Targeted Therapeutic Strategies

Getting the most of CFTR-France to classify variants and complex alleles in the CFTR gene

Caroline Raynal

Molecular diagnosis of cystic fibrosis (CF) and *CFTR*-related disorders (CFTR-RDs) has identified over 2,100 sequence variations in the *CFTR* gene worldwide. CF and particularly *CFTR*-RD provide a particular challenge for molecular diagnostic because of the increasing number of variants of unclear significance (VUS) and the phenotypic variability reported for identical genotypes. *CFTR*-France, a database dedicated to the annotations of rare *CFTR* variants, was built thanks to the collaboration of 10 French laboratories experts in the *CFTR* gene analysis and the French Cystic Fibrosis Registry. *CFTR* variants are annotated in the context of their *cis*- and *trans*-allelic combinations, associated to patients' phenotypic data.

So far, 1046 different variants have been collected from 5962 individuals, including individuals affected with CF or *CFTR*-RD such as congenital absence of vas deferens (CBAVD), bronchiectasis, chronic pancreatitis, chronic rhinosinusitis, aquagenic palmoplantar keratoderma, but also asymptomatic compound heterozygous individuals, fetuses with ultrasound bowel anomalies and newborns awaiting clinical diagnosis (CFSPID).

About 70% of all variants have been reported three times or less in the database and 33% are still of unknown significance. Combining genetic data with clinical, epidemiological and functional data thus enables a more detailed analysis of variations, haplotypes, complex alleles and associated phenotypes. To assist with the interpretation of undocumented variants, the website also provides access to two bioinformatics tools developed in-house: CYSMA (CYStic fibrosis Missense Analysis) (Sasorith et al. 2020) and Mobidetails (Baux et al. 2020). Finally, we have recently added a section dedicated to the available data (from medicines agencies and publications) on how variants respond to CFTR modulators.

During this presentation, I will show how to query the database and, using various examples, what information is available and how it can be useful in clinical practice and genetic diagnosis.

CFTR Complex Alleles in Individuals with Cystic Fibrosis Showing Limited Response to Elexacaftor/Tezacaftor/Ivacaftor (ETI) Triple Modulator Therapy

Maike Karnstedt, Katharina Schütz, Simon Gräber, Jasmin, Berger, Mirjam Stahl, Jutta Hammermann, Susanne Hämmerling, Anette Scharschinger, Hana Isijanov, Annika Geppert, Simone Ahting, Julia Hentschel

Introduction: In many people with cystic fibrosis (pwCF), the diagnosis is established genetically, often through analyses that detect only common CFTR gene variants. In particular, so-called complex alleles - multiple variants occurring on the same allele - frequently remain undetected. These can influence CFTR protein function, response to modulators such as elexacaftor/tezacaftor/ivacaftor (ETI) and, consequently, disease progression.

Methods: The use of next-generation sequencing (NGS) is therefore crucial to identify previously unknown variants and to determine the phase of variants (in cis/in trans). A deeper understanding of CF's genetic diversity forms the basis for advancing personalized therapies. The aim of our study is to investigate whether individuals with a reduced response to ETI - defined by a limited decrease in sweat chloride (<10–15 mmol/L) - carry complex alleles and to further characterize these alleles. All known complex alleles are being investigated using NGS, with particular attention to L467F, which has been shown to exert a regulatory effect on ETI. However, since the frequency and functional role of other complex alleles remain unclear, NGS will be used to comprehensively screen for all of them.

Results: In our pilot study, we recruited samples from 39 cases from participating CF centers, including 16 samples from high responders who respond well to ETI (defined by a decrease in sweat chloride of more than 40 mmol/L after initiation of ETI) and 11 cases with a lower-than-expected response (low responders). The remaining 12 pwCF show sweat chloride reductions in response to ETI of 15-40 mmol/L and were therefore defined as intermediate responders. Preliminary analyses of the low responders revealed that 8 of the 11 individuals examined so far carry a complex allele, seven of whom harbor [Phe508del; Leu467Phe] and one a yet undescribed complex allele [Phe508del; c.1585-9412A>G]. All of the intermediate responders do not carry a complex allele.

Conclusion: We plan to recruit approximately 50 individuals with CF for whom the CFTR genotype will be analyzed using NGS to assess the presence of complex alleles. In the long term, this analysis aims to deepen our understanding of the genetic heterogeneity of cystic fibrosis in Germany, particularly regarding complex alleles. The findings are expected to provide a foundation for future projects in personalized therapy for people with CF and, prospectively, to be implemented in routine diagnostics.

Effectiveness of ETI in CF patients with complex alleles of the CFTR gene

Tatjana Jakjovska Maretti

Objectives: Cystic fibrosis (CF) is an autosomal recessive disease caused by pathogenic variants within the CFTR gene. Complex alleles arise as a result of a combination of two or more CFTR variants in the cis position on the same allele. Variants of p.Leu467Phe and p.Phe508del have an additive pathogenic effect to the effectiveness of targeted therapy.

Aim: To examine the frequency of p.[Leu467Phe;Phe508del] complex allele in PwCF in North Macedonia with a F508del genotype and its effect on the clinical course of cystic fibrosis and the effectiveness of triple therapy.

Method: Sequencing of coding regions of CFTR gene and analysis of polymorphic markers in 76 PwCF carrying F508del variant and comparing the clinical features between patients having genotypes [L467F;F508del];[F508del] and [F508del; L467F];[F508del; L467F].

Results: By sequencing the CFTR gene, it was found presence of complex allele [L467F; F508del] in 8 CF patients (10, 5%); 3 CF patients (4%) have homozygous [L467F; 508del] complex alleles. When comparing the examined groups with homozygous F508del and compound heterozygous F508del and [L467F; 508del], we did not detect a statistically significant difference in the course of the disease, exacerbations, or prescribed therapy.

Conclusion: The frequency of complex alleles associated with F508del was found in 10, 5% in CF patients in North Macedonia, which should be taken into account for the decision on optimal treatment options with CFTR modulators. The presence of an additional amino acid replacement, L467F, in a cis state with F508del leads to a significant decrease in CFTR activity due to a serious maturation defect and poor effect of ETI therapy.

Keywords: CFTR, Cis-mutation, Complex alleles, Cystic fibrosis, ETI

Targeted Nanopore Sequencing as a Key to Improving CF Diagnostics: Characterization of Splicing Variants to Increase Diagnostic Yield

Simone Ahting, Maike Karnstedt, Patricia Duffek, Stephan Drukewitz, Denny Popp, Julia Hentschel

Introduction: Treatment of cystic fibrosis (CF) with highly effective modulators requires precise knowledge of CFTR genetic variability. Splicing-affecting variants are often poorly characterized, leaving their functional impact and mutational class - particularly the presence of residual CFTR function - uncertain. Since this classification is essential for modulator eligibility, unresolved cases pose a major clinical challenge. Rare intronic CFTR variants further complicate diagnostics, as their effect on mRNA or protein is frequently unknown, leading to inconclusive results in people with suspected CF (pwCF). To improve detection of such variants, our group previously established a Next-Generation-Sequencing (NGS) panel covering the entire CFTR locus. We now build on this by using targeted long-read mRNA sequencing to characterise the splicing impact of NGS-detected intronic variants and to infer the most likely mutational class, thereby improving diagnostic confidence and informing therapy eligibility.

Methods: CFTR mRNA was isolated from nasal epithelial swabs of pwCF harbouring suspected splicing variants with unknown impact. The region harbouring the predicted splice effect was selectively amplified by targeted PCR, and the resulting amplicons were analysed using targeted Nanopore long-read sequencing. To validate the method, we sequenced samples with known splicing variants as well as control samples without CFTR variants, demonstrating physiological CFTR splicing. This approach enables direct detection and interpretation of splicing alterations—such as exon skipping or inclusion—allowing functional assessment of potentially pathogenic intronic CFTR variants.

Results and Conclusions: Of the 68 pwCF in whom NGS panel analysis remained inconclusive despite a clinical suspicion of CF, nasal epithelial samples were available for 30 individuals, covering a range of diagnostic scenarios. Analysis of the first 11 samples revealed disease-relevant aberrant splicing events in three individuals with previously undescribed intronic splicing variants (c.1584+675A>G, c.3874-247A>G, c.2908+1211T>G), confirming CF diagnosis and enabling the pwCF to receive targeted modulator therapy. One variant showed no splicing effect, excluding CF (c.1680-87T>G). A complex allele (c.[1521_1523delCTT; c.1585-9412A>G]) showed an unexpected splicing pattern with two pseudoexons, while c.489+3A>G caused an in-frame splicing alteration in a healthy proband with R553X on the other allele, confirming previous reports describing this variant as non-CF-causing. Three variants remain under investigation (c.2989-2A>G, c.3964-7A>G, c.53+1005T>G), while a p.(Glu528=) homozygous individual showed partial exon 11 elongation (+85 bp) without exon skipping, supporting prior data and a likely non-classic phenotype.

Discussion: Our results demonstrate that combining extended DNA diagnostics - including intronic regions - with long-read mRNA sequencing represents an important addition to the diagnostic workup of unclear CF cases. This underscores the importance of comprehensive molecular genetic analysis for precise diagnosis and broader access to modulator therapies.

Transcriptomic analysis of G542X-CFTR bronchial epithelial cells indicates inhibition of nonsense mediated decay by IL-4 treatment

Anna Borrelli, Arianna Venturini, Martina De Santis, Rossella De Cegli, Enza Montemitro, Federico Alghisi, Fabiana Ciciriello, Luis J.V. Galietta

Background: Premature termination codons (PTCs) due to nonsense mutations in the CFTR gene hamper protein synthesis. The resulted truncated forms of the CFTR chloride channel are insensitive to the available therapies. Ribosome read-through (RT) by small molecules is a promising way to restore CFTR synthesis. ELX-02 is indeed a RT agent currently in clinical trial for people with cystic fibrosis. Small molecules acting as eRF3a degraders, such as CC-90009, also emerged as effective on PTCs. Inflammatory stimuli can profoundly change the transcriptome and proteome of airway epithelial cells, and it has been reported that TNFα and IL-17A enhance the efficacy of CFTR modulators in rescuing F508del-CFTR in human bronchial epithelial cells (HBECs). Recently we reported that in vitro cultured HBECs showed an enhanced response to a triple compound combination including VX-809 as CFTR corrector, ELX-02 as a RT agent and the eRF3a degrader CC-90009 when treated for 72 hours with IL-4 or TNFα plus IL-17. We are currently investigating the mechanism by which cytokine stimulation together with drug treatment enhance CFTR function at the molecular level.

Methods: In vitro differentiated HBECs from a patient with the G542X mutation were cultured ± IL-4 or TNFα/IL-17A for 72 h. In the last 24 h, cells received ELX-02 + VX-809, with or without CC-90009. CFTR function was measured by short-circuit current, and protein and mRNA levels by capillary immunoblot and qRT-PCR, respectively. Bulk RNA-seq assessed gene expression changes under treatments.

Results: Combination of CC-90009, ELX-02, and VX-809 (combo) led to a 3-fold increase in CFTR current. Cytokine treatment significantly improved this effect, with a 15-fold increase in presence of IL-4 and 9-fold with TNFα/IL-17A. Improved function mirrored increased full-length CFTR protein and mRNA levels. We focused on IL-4 given its strong effect on full-length CFTR expression. Reduced SMG6 and UPF1 protein expression suggest that cytokine may limit nonsense mediated decay (NMD) process. Gene-set enrichment analysis (GSEA) of RNA-seq data showed significant downregulation of the reactome terms “nonsense mediated decay pathway”, “rRNA processing”, “response of EIF2AK4-GCN2 to amino acid deficiency” in epithelia treated with IL-4 alone compared to control condition. The same datasets appear downregulated also in epithelia treated with the combo alone and in epithelia where IL-4 was added to the combo, but the single downregulated genes contributing to the pathway were different. In particular, IL-4 contributes to the downregulation of the NMD pathway decreasing UPF3A and PABPC1 transcripts. Interestingly, the reactome terms “Unfolded protein response (UPR)” and “ATF4 activates genes in response to endoplasmic reticulum stress” appears upregulated after GSEA in epithelia treated with the combo and the combo plus IL-4, but not in epithelia treated with IL-4 alone.

Conclusions: Transcriptomic analysis of G542X-CFTR bronchial epithelial cells suggests inhibition of NMD by IL-4 treatment. A deeper investigation is needed to reveal novel molecular targets belonging to translation fidelity, ribosome quality control and post-translational modifications pathways that can be modulated with other pharmacological strategies.

Funding: Cystic Fibrosis Foundation (GALIET22I0), Italian Cystic Fibrosis Foundation (FFC#9/2022, FFC#4/2025), and Italian Ministry of Health (GR-2018-12367126).

Identification of novel pharmacological inhibitors of the nonsense-mediated RNA decay mechanism to rescue CFTR function in patients carrying nonsense mutations

Arianna Venturini, Anna Borrelli, Fabiana Ciciriello, Luis J.V. Galietta

Introduction: Nearly 10% of CFTR mutations, particularly premature termination codons (PTCs), are insensitive to CFTR modulators (i.e., correctors and potentiators). The pharmacological rescue of PTCs requires a combinatorial approach that includes inhibitors of the nonsense-mediated RNA decay (NMD) mechanism, essential to increase the amount of PTC-CFTR transcripts, as well as compounds that promote the ribosomal readthrough. NMD inhibitors can also be effective in the absence of a readthrough agent for PTCs localized at the carboxy-terminus of the CFTR sequence such as W1282X. The aim of our project was to identify novel molecules able to modulate the NMD mechanism.

Methods: We screened a chemical library of more than 9,000 compounds, including drugs, investigational drugs, and compounds with known biological activity (mechanistic probes), using the 16HBE14o– cell line expressing W1282X-CFTR (obtained from CFF) and the halide-sensitive yellow fluorescent protein (HS-YFP). Cells were incubated for 24 with test compounds plus CFTR correctors. The functional assay allowed to identify compounds enhancing CFTR function. The most effective molecules were characterized by secondary biochemical and functional assays to assess their mechanism of action.

Results: 75 primary hits were identified by the screening. Molecules with largest effect on CFTR rescue were further tested in the presence/absence of CFTRinh-172 to confirm a direct activity on CFTR channel and not on other anion channels/transporters. We focused our attention on three different compounds: CC-115, samotolisib and elimusertib. Interestingly, all of them are known kinase inhibitors. They induced more than 10-fold increase in CFTR-mRNA transcripts and promoted the appearance of a signal for CFTR protein. CC-115 and samotolisib, together with CFTR correctors, generated a marked increase in CFTR channel activity in short-circuit current recordings experiments. Elimusertib per se elicited a significant increase in CFTR function that was further amplified when it was combined with correctors. We also conducted a deep bioinformatic analysis on 16HBE14o- cells, both the wild type and W1282X versions, to assess their effects on whole cells transcriptome. We were able to highlight all the substrates that are subjected to NMD, as well as common and compound-specific signatures elicited by treatments. We found CFTR among the most commonly upregulated genes in W1282X cells, together with several NMD-related factors. These results confirm that CC-115, samotolisib and elimusertib share a similar mechanistic behaviour in modulating the NMD pathway and a specific action in increasing PTC-CFTR transcripts.

Conclusions: We have identified three possible candidates able to modulate NMD mechanism and improve the extent of CFTR rescue in cystic fibrosis patients with PTCs.

This project was supported by CFF (GALIET22I0) and the ECFS (post-doctoral research fellowship to Arianna Venturini)

Session II: Precision Therapeutics: Functional Profiling and Next-Generation Modulators

Leveraging Single-Cell RNA Sequencing to Reveal Tissue-Specific Effects of ETI Therapy in Pediatric Cystic Fibrosis

Saskia Trump

Highly effective CFTR modulator therapy with elexacaftor/tezacaftor/ivacaftor (ETI) has transformed the clinical management of cystic fibrosis (CF), leading to substantial improvements in lung function and overall outcomes. However, its impact on mucosal homeostasis and host defense at the molecular and cellular level, particularly in children, remains incompletely understood. The recent application of single-cell RNA sequencing as a novel, high-resolution technology enables detailed characterization of these processes across distinct epithelial and immune cell populations.

We applied single-cell RNA sequencing to nasal swab samples from children with CF collected at baseline and after three months of ETI therapy and compared them with age-matched healthy controls. We further extended our single-cell approach to matched rectal samples from children with CF collected at baseline and after the same three-month ETI follow-up.

In the nasal samples at baseline, children with CF exhibited impaired interferon signaling, and decreased expression of major histocompatibility complex (MHC) class I and II genes. Immune cells displayed a pronounced pro-inflammatory phenotype, particularly among neutrophils and macrophages. Following ETI initiation, epithelial immune pathways partially recovered, and inflammatory signatures in immune cells were markedly reduced.

While ETI also improved cellular homeostasis in the rectum, the molecular responses were distinct from those in the airway—consistent with the fundamentally different biology of these epithelial barriers—highlighting tissue-specific effects of CFTR modulation.

Overall, our findings demonstrate that ETI therapy promotes restoration of epithelial and immune homeostasis at single-cell resolution in children with CF, supporting early intervention and illustrating the power of single-cell technologies to resolve tissue-specific therapeutic responses.

Expanding the therapeutic landscape: Vanzacaftor-Tezacaftor rescues orphaned ETI-resistant genotypes

Valeria Capurro, Emanuela Pesce, Federico Cresta, Cristina Pastorino, Valeria Tomati, Angelica Quarato, Alice Mantero, Alice Dighero, Raffaele Pisani, Carlo Castellani, Nicoletta Pedemonte

Background: The triple combination of elexacaftor/tezacaftor/ivacaftor (ETI) has revolutionized cystic fibrosis (CF) treatment; however, a subset of patients with eligible genotypes remains unresponsive. Notable among these are the L467F-F508del complex allele, which confers a severe processing defect refractory to ETI, and rare missense variants such as H147P and I507del. To address this unmet need, we evaluated the efficacy of the next-generation correctors combination comprising vanzacaftor and tezacaftor (VT) in rescuing these ETI-resistant phenotypes.

Methods: We utilized primary human nasal epithelial (HNE) cells derived from people with CF (pwCF) carrying a class I variant in combination with an ETI-non-responsive variants, including six patients with the L467F-F508del complex allele, 3 pwCF with the H147P variant, and 3 pwCF with the I507del. CFTR-mediated chloride transport was assessed via short-circuit current analysis in Ussing chambers following treatment with ETI or VTI. Mechanistic validation was performed in heterologous expression systems (CFBE41o- cells). Additionally, we monitored the clinical response of a patient with the L467F-F508del genotype treated with VTD via a compassionate use program.

Results: In patient-derived HNE models, VTI demonstrated superior corrective potency compared to ETI. For the L467F-F508del complex allele, treatment with the vanzacaftor/tezacaftor combination resulted in significant rescue of chloride transport, increasing inh-172-sensitive currents up to 3.0-fold compared to vehicle control. This rescue restored CFTR activity to approximately 15–25% of wild-type levels, overcoming the block previously observed with ETI. Biochemical analysis in heterologous systems confirmed that Vanzacaftor/Tezacaftor successfully promoted the formation of the mature, complex-glycosylated Band C form of the protein, whereas ETI failed to produce a detectable mature species. This ex vivo efficacy was validated clinically, with the treated patient experiencing a rapid improvement in pulmonary function (FEV1 +120mL) and exercise tolerance. Similarly, for the H147P variant, treatment with VTI increased baseline current up to 3.5-fold, reaching levels corresponding to > 30% of wild-type activity. Functional and biochemical analyses for the I507del variant are currently ongoing.

Conclusions: Our findings demonstrate that the vanzacaftor/tezacaftor combination possesses superior efficacy capable of rescuing the severe trafficking defects of variants currently orphaned by standard-of-care modulators. VTD represents a promising therapeutic solution for ETI-resistant variants, highlighting the utility of integrating ex vivo screening with clinical monitoring to define precision medicine strategies for difficult-to-treat genotypes.

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

Jasmin Berger, Anita Balázs, Tihomir Rubil, Alicia González, Julian Berges, Yin Yu, Rebecca Dalferth, Katharina Schütz, Rory E. Morty, Anna-Maria Dittrich, Olaf Sommerburg, Mirjam Stahl, Marcus A. Mall, Simon Y. Graeber

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

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

Results: 37 people with CF showed no increased CFTR activity in pHNEC after ETI treatment compared to DMSO. However, 17 people with CF and non-F508del mutations showed ETI response with a mean correction of CFTR activity of 20% of the healthy level. 12 people with CF who received ETI therapy showed a mean improvement in FEV1% predicted by 12%. All 12 patients showed a pathological SCC above 60 mmol/L at baseline, which was reduced by -36 mmol/L after initiation of ETI ($p < 0.001$). In the ICM, the cAMP-dependent chloride secretory response increased from -6 µA/cm2 at baseline to 98 µA/cm2 after initiation of ETI ($p < 0.001$) and the total chloride secretory response increased from -18 µA/cm2 to 130 µA/cm2 ($p < 0.001$). A subgroup of 7 patients, where NPD was performed, showed an increase in total chloride response from 2.8 mV at baseline to -12 mV after initiation of ETI in the nasal epithelium.

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

Omics to understand the side effects of CFTR Modulators

Angelica Squarzoni, Gaia Boschetti, Sine Mandrup Bertozzi, Maria Summa, Angelo Serani, Elisa Milandri, Roberto Mandrioli, Michele Protti, Laura Mercolini, Caterina Montani, Giovanna Capodivento, Giuliana Cangemi, Nicoletta Pedemonte, Tiziano Bandiera, Fabio Benfenati, Lucilla Nobbio, Rosalia Bertorelli, Andrea Armirotti

Introduction: Over the last years, we used proteomics, lipidomics and metabolomics to explore both on-target and off-target effects of CFTR modulators. By using spatial proteomics, we investigated the mitochondrial reorganization of F508del bronchial epithelial cells associated with the treatment with VX-809 (1). More recently, we moved to the triple combination ETI and we demonstrated (2,3) that Tezacaftor inhibits the enzyme (DEGS) which converts dihydroceramides (dHCer) into ceramides (3), thus producing increase of dHCer in various cells and tissues. Since DEGS malfunctioning is often associated with severe neurological impairments linked with aberrant myelin composition and structure (4,5), we conducted an in vivo drug safety study, by administering ETI to CD-1 mice during pregnancy and breastfeeding. The aim of this work is to evaluate the potential impact of DEGS inhibition by ETI on the formation of the peripheral and central nervous systems (PNS, CNS).

Methods: ETI was incorporated as powder into mouse food, in a high-fat diet regimen. Besides recording weight and size, we also investigated pups' behavior by means of dedicated tests. ETI and dHCer levels in plasma and tissues, as well as changes in the global lipidome were measured by tandem mass spectrometry coupled to liquid chromatography.

Results and Conclusions: At 10 days after birth, we observed a significant accumulation of dHCer in the brains of pups born from ETI-fed dams compared to controls. No accumulation was observed in the sciatic nerve of these animals, likely due to much lower levels of ETI in this tissue compared to the brain. We also conducted an untargeted lipidomics survey, which revealed other alterations in lipid metabolism associated with exposure to ETI during pregnancy. During breastfeeding, when exposure to the drug decreases, these alterations revert and virtually disappear at P28, together with other differences in the phenotype and behaviour of the pups observed earlier during development (6). Despite a few experimental difficulties, which we are overcoming using also a bit of creativity, our project is progressing toward a better understanding of the practical implications of these findings.

Acknowledgements: The support of Cystic Fibrosis Foundation (grant ARMIRO24G0 to AA) and Fondazione per la Ricerca sulla Fibrosi Cistica – Onlus (grant FFC#2-204 to LN) is gratefully acknowledged.

References: (1) Braccia et al. DOI: 10.3390/cells11121938 (2) Liessi et al. DOI:10.1016/j.jcf.2023.04.012 (3) Ciobanu et al. DOI:10.1016/j.jcf.2024.05.004 (4) Fabrias, et al. DOI:10.1016/j.plipres.2011.12.002 (5) Karsai, et al. DOI: 10.1172/JCI124159 (6) Pant, et al. DOI: 10.1172/JCI123959 (7) Doretto et al. DOI:10.1371/journal.pone.0019849 (8) Squarzoni et al. DOI: 10.1016/j.jcf.2025.11.004

Keynote Session

Proteostasis landscapes of rare CFTR variants to emerging correctors and off-targets of Elexacaftor/Vanzacaftor

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Cystic fibrosis therapy has been revolutionized over the last decade by the availability of corrector and potentiator small-molecule drugs, combined into highly effective modulator therapies. While correctors were initially developed for F508del-CFTR, the most common mistrafficking variant, corrector combinations are now approved to treat a broad range of CFTR genotypes, including rare variants. Despite their widespread success, several challenges remain. First, correctors do not equally restore the folding and trafficking of all misfolding-prone CFTR variants, particularly poorly responsive variants such as those harboring mutations localized to nucleotide-binding domain 1. With recently approved correctors (e.g., vanzacaftor/VX-121) and emerging candidates under development, it will become increasingly important to match CFTR variants in people with CF to their most effective correctors. This underscores the need for high-throughput in vitro characterization of therapeutic responses (theratypes) and a deeper mechanistic understanding of the cellular determinants that drive the theratype. A second challenge is the adverse effects associated with modulator drugs – particularly neuropsychiatric effects – whose underlying molecular mechanisms remain poorly understood.

Research in our lab combines mass spectrometry and chemical biology approaches to profile dynamic protein interaction networks. We have developed spatial and temporal interactomics approaches to study membrane protein-misfolding diseases, with a particular focus on CFTR, related ABC transporters, and voltage-gated potassium channels. Here, I will present our latest research characterizing the protein quality control interaction networks (proteostasis landscapes) of dozens of CFTR variants – many of which are poorly responsive to approved corrector therapies. We reveal how responsive and unresponsive variants diverge in their engagement with protein quality control surveillance machinery. Complementary structural modeling of CFTR variants can highlight specific domain interfaces at which correctors fail to confer sufficient conformational stability in poor responders. We have leveraged these insights into structural and proteostasis vulnerabilities to evaluate new therapeutic strategies for poorly responsive CFTR variants not addressed by existing drugs. Deep mutational scanning of CFTR cell-surface expression has further enabled us to evaluate the therapeutic responses of 232 variants to existing and investigational macrocyclic corrector drugs. For example, when comparing the structural analogs VX-445 (elexacaftor) and VX-121 (vanzacaftor), we found that VX-121 generally conferred greater CFTR cell-surface expression, though the effect size varied considerably across individual variants. Finally, I will discuss our chemoproteomics studies evaluating off-target engagement of VX-445 and related correctors using a functionalized photoaffinity-labeling probe.

Session III: Targeting the Airway Interface: Microbial Persistence, Immunity, and Clinical Outcomes

Integrative computational analysis of longitudinal effects of Elexacaftor/Tezacaftor/Ivacaftor on sputum microbiome and proteome in patients with cystic fibrosis

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Objective: Despite beneficial effects of CFTR modulator therapy with elexacaftor/tezacaftor/ivacaftor (ETI) on the airway microbiome and inflammation in patients with CF, residual airway infection and inflammation persist at a level expected to contribute to progressive lung damage over time. The aim of this project was therefore to initiate an integrative computational analysis to identify novel biomarkers and therapeutic targets for persisting airway infection and inflammation.

Methods: Sputum samples were collected from CF patients with at least one *F508del* allele (≥ 12 years) prior and 3 months on ETI. Patients were classified as responders and low responders to treatment based on change in lung function and sweat chloride concentration. An integrative data analysis was used to determine relations between clinical, mucus, proteomic, inflammatory and microbial measures in responders vs. low responders.

Results: Responders showed improvement in mucus properties, inflammation and airway microbial composition compared to low responders. In low responders, *Pseudomonas* correlated positively with inflammation markers, sputum elasticity and negatively with mesh-pore size (pFDR<0.001) and was highly increased in CF patients taking inhaled antibiotics. We identified 70 deconfounded proteins associated with time of treatment showing differential patterns in responders vs. low responders, mainly linked to immune cell function and host defense.

Conclusions: Our integrated multi-omics analysis provides first insights into bacteria-host associations on ETI therapy with distinctive protein association patterns in responders vs. low responders. Potential candidate biomarkers or therapeutic targets associated with residual inflammation in CF patients on ETI require further validation.

Characterization of mucin glycan structures

Gaël Vos, Jacob S. Jordan, Kevin Pagel

Mucins are heavily glycosylated polymers that define the biophysical and protective properties of airway mucus, yet their glycan architectures remain incompletely resolved. We develop methods to rapidly assess glycosylation and find clear glycosylation changes in the sputum of cystic fibrosis (CF), where altered mucus composition contributes to impaired clearance and chronic infection.

By combining advanced mass spectrometry with ion mobility spectrometry (IMS), we can resolve isomeric glycans which have identical composition but differ in their 3D structure. We will highlight our recent developments in high-throughput glycomic analysis that enable the rapid profiling of mucin glycans. A particular focus will be given on modified residues that are so labile that they are fully lost during sample preparation but play a critical role in regulating the mucosal interactome.

Deficiency of a repressor of maltodextrin metabolism in commensal *Streptococcus* correlates with *Pseudomonas aeruginosa* growth inhibition

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Introduction: Commensal *Streptococcus* isolates have shown antiinflammatory and antipseudomonal effects, offering new approaches to prevent pulmonary decline in people with cystic fibrosis (pwCF), mostly caused by *Pseudomonas aeruginosa (Psae)*. However, *Streptococcus* strains in the airways of pwCF may have co-evolved with pathogens, reducing their virulence in bacterial competition. This project evaluated the potential competitiveness of commensals from people with and without CF against *Psae*.

Methods: 29 *Streptococcus mitis/oralis* strains were isolated from respiratory samples of both cohorts. Growth capacities were analysed, and capacities to inhibit the *Psae* strain PAO-1 were tested in two competition assays. Genotypes associated with *Psae* inhibition were identified using Genome-wide association studies (GWAS).

Results: Five isolates inhibited PAO-1 growth in both competition assays, and ten in at least one assay. The amount of inhibiting *Streptococcus* isolates did not significantly differ between CF and non-CF cohorts. Multivariate GLM revealed *Streptococcus* isolates with an in vivo contact with *Psae*, and those showing stronger growth in monoculture were more likely to inhibit PAO-1. GWAS revealed a significant association between PAO-1 inhibition and a deficiency of the malR gene in *Streptococcus* isolates.

Conclusions: The *Psae* inhibiting effects seem to be strain-specific rather than CF dependent. Further research is needed to clarify how the commensals' growth capacities and *Psae*-induced commensal selection influence the bacterial interplay. As malR repression in *Streptococcus* was previously linked to enhanced maltodextrin utilization, comparing malR-deficient and wild-type *Streptococcus* strains will help to validate the results of this project and advance the development of non-antibiotic therapies for the CF lung disease.

Characterization of mucoid hyper biofilm forming *Staphylococcus aureus* isolates recovered from the airways of a person with cystic fibrosis with a double mutation in the ica operon

Christine H. Rumpf, Benedikt J. de Sousa, Susanne Deiwick, Barbara C. Kahl

Introduction: The most commonly isolated pathogen in the airways of people with cystic fibrosis (pwCF) is *Staphylococcus aureus (SA)*. *SA* is able to persist for long periods despite antibiotic therapy due to various adaptational mechanisms including biofilm formation. In a prospective multicenter study, mucoid *SA* isolates with hyper biofilm formation were detected in airway specimens of pwCF. In approximately 30% of the mucoid isolates, a 5-base pair deletion (5bp-del) was detected in the intergenic region (IGR) of the intercellular adhesin (ica) operon, which is responsible for the production of polysaccharide-dependent biofilm (PIA). In addition to the 5bp-del mutation, we also identified mutations in the repressor gene icaR of the ica operon in approximately 30% of the study isolates. To date, all mucoid isolates in this study have shown single mutations, either the 5bp-del or a mutation in icaR. However, in one pwCF, a 5bp-del was found for the first time together with a simultaneous mutation in icaR. Such a functional double mutation in the ica operon has not been described before. Therefore, the aim of this study was to characterize these strains intensely to determine a potential advantage or increased virulence.

Methods: *SA* isolates (n=40) from 4 visits during a prospective multicenter study were available for analyses. Genetic relatedness between the bacterial isolates was determined by spa typing. Whole genome sequences of selected isolates were compared in terms of their mucoid biofilm-forming phenotype. Biofilm formation was assessed in a micro titer plate assay and further characterized in enzymatic detachment reactions. The fitness of bacterial isolates with mucoid/non-mucoid phenotypes was quantified in competitive growth assays.

Results: During the first visits, all mucoid and non-mucoid isolates belonged to the same spa type, while at the last visit non-mucoid isolates belonging to a different spa type occurred. All mucoid isolates produced high amounts of biofilm, which was polysaccharide-dependent. Double mutations (5-bp-del/icaR) in mucoid isolates occurred in all 4 visits in different quantities. The icaR mutation was also found in non-mucoid isolates in addition to a compensatory mutation in one of the ica synthesis genes. While mucoid and non-mucoid isolates did not differ in their growth kinetics, the mucoid isolates outcompeted the non-mucoid isolates in co-culture.

Discussion: Interestingly, such a double mutation within the ica operon seems to be stable since isolates carrying these mutations were recovered from all visits during the one-year period. Surprisingly, the mucoid isolates outcompeted the non-mucoid isolates indicating a survival advantage conferred by these mutations.

Conclusions: There are different possible mutations (5bp-del or mutations in icaR) observed within the ica operon of *SA* causing hyper biofilm formation. Within this particular pwCF, special environmental conditions seem to induce such hyper biofilm causing mutations in a most likely timely dependent fashion, which are beneficial for survival of these double-mutated strains.

IL-17A and IL-17F differentially modify transcriptomic profile of human bronchial epithelia

Daniela Guidone, Martina De Santis, Chiara Ferrari, Francesca Nicola, Nicola I. Lorè, Luis J.V. Galietta

Background: The airway epithelium deploys innate defense mechanisms that have a key protective role against pathogens. The mucociliary clearance (MCC) process is controlled by a fine balance of the airway surface properties. In cystic fibrosis (CF), the absence of chloride secretion through CFTR leads to dehydration of the airway surface, MCC impairment, bacterial colonization, persistent inflammation and progressive lung damage. IL-17 cytokines, particularly IL-17A and IL-17F, are involved in the immune response to extracellular bacteria and the recruitment of neutrophils in CF lung disease. These cytokines interact with IL-17 receptors, IL-17RA and IL-17RC, that can form both homodimers and heterodimers with different affinity for the two cytokines. In particular, IL-17A binds preferentially the RA-RA homodimer, while IL-17F the RC-RC homodimer.

Methods: We compared the effects of IL-17A, IL-17F or IL-17A plus IL-17F on bronchial epithelia by monitoring transepithelial ion transport with short circuit current recordings. While both cytokines increase CFTR function, only IL-17A upregulates ENaC activity. We also included brodalumab, a neutralizing antibody against IL-17RA. Brodalumab blocked the upregulation of ENaC and CFTR generated by IL-17A, but did not prevent the upregulation of CFTR caused by IL-17F.

Results: We analyzed the gene expression changes induced by IL-17A, IL-17F and the combination of the two cytokines. Among the genes upregulated by IL-17A there are granulocyte stimulating factor CSF3, the SLC26A4 anion exchanger, the cytokine IL19, the defensin DEFB4A, the sodium/monocarboxylate cotransporter SLC5A8, the sodium/glucose cotransporter SLC5A1, the chemokines CCL20 and CXCL3, the transcription regulator NFKBIZ, the beta-adrenergic receptor ADRB2, and the kinase SGK1. These genes were similarly induced by the combination IL-17A/IL-17F. Instead, they were less affected by IL-17F. Upregulation of these genes was largely prevented by brodalumab. Instead, the genes upregulated by IL-17F are the cystatins CST1 and CST4, the matrix protein POSTN, the chemokine CCL26, the inducible nitric oxide synthase NOS2, the calcium-activated chloride channel ANO1, and the suppressor of cytokine signaling CISH. Upregulation of these genes was not inhibited by brodalumab.

Conclusion: In conclusion, IL-17F/IL-17RC signaling prevents ENaC upregulation and increases chloride secretion through CFTR and ANO1, promoting hydration of the airway surface. These findings support the targeting of IL-17A signaling while preserving effects driven by IL-17F, using brodalumab as a therapeutic agent. However, possible side effects of IL17RA inhibition should be considered.

Linking highly neutralizing human antibodies targeting multidrug-resistant *Pseudomonas aeruginosa* to clinical and immunological phenotype in people with cystic fibrosis

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Introduction: Multidrug-resistant *Pseudomonas aeruginosa (PA)* poses an emerging threat to human health with urgent need for novel therapeutic approaches. In people with cystic fibrosis (pwCF), *PA* frequently establishes chronic infections in the lung. We previously demonstrated that repeated exposure to *PA* can elicit highly neutralizing antibodies directed against its type III secretion system (T3SS). However, the immunological dynamics underlying chronic infection and the clinical significance of these antibodies remain poorly understood.

Methods: We integrated clinical data and serum T3SS-neutralizing activity from 99 pwCF and 12 healthy individuals with immunophenotyping of immune cell populations and bulk RNA sequencing of peripheral blood mononuclear cells (PBMCs).

Results: As expected, prolonged *PA* infection was associated with reduced lung function. Although chronic infection correlated with the presence of high titers of T3SS-neutralization antibodies (PA-Abhigh), these antibodies did not confer measurable protection against lung function decline, consistent with previous findings that the T3SS plays a subordinate role in chronic lung infections. Importantly, comparison of pwCF with high versus low neutralization capacity revealed distinct immune signatures: Flow cytometry demonstrated altered $CD8^+$ T-cell profiles in PA-Abhigh individuals, marked by expansion of terminal effector subsets and reduction of naïve $CD8^+$ T cells. These cellular alterations were mirrored in transcriptomic differences, particularly within cytokine–cytokine receptor interaction and adaptive immunity pathways.

Conclusion: Our findings provide mechanistic insight into the development of highly neutralizing anti-T3SS antibodies and identify a distinct immunological profile associated with chronic *PA* infection and impaired lung function. Incorporating this immune signature into clinical practice may support the early identification of patients at risk of disease progression.

Session IV: Developmental and Epigenomic Network Dynamics in Airway Disease

Microbial and fungal dynamics in the respiratory tract of infants with cystic fibrosis

Ruth Steinberg

Upper airway microbiome alterations in infants with cystic fibrosis (CF) occur early in life and are associated with poorer respiratory outcomes. However, the impact of early-life therapeutic interventions on microbial adaptation remains poorly understood.

Using longitudinal nasal swab samples from infants with CF and healthy controls from two cohort studies, we combined metagenomic and 16S rRNA sequencing to characterize early microbial airway dynamics and construct a comprehensive infant nasal microbiome gene atlas. We further investigated the effects of inhaled hypertonic saline therapy on airway microbial composition and function.

We found that early hypertonic saline inhalation leads to enrichment antibiotic resistance-associated transporter genes, and promotes expansion of opportunistic bacteria and fungi.

Restoring near-normal F508del-CFTR function: a cell-permeable nanobody synergizes with CFTR modulators in primary airway epithelial cells from patients with cystic fibrosis

Tihomir Rubil

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

Methods: We synthesized a cysteine-containing NB1 and conjugated it to a CPP (R10) via a disulfide bond. CFBE41o- cells expressing F508del-CFTR were treated with NB1-R10, and uptake of fluorescently labeled NB1-R10 was assessed by live-cell microscopy and Western blot. Dose-response studies on the effect of NB1-R10 on F508del-CFTR function were conducted using transepithelial short circuit current (Isc) measurements, quantifying CFTR inhibitor-172-sensitive Isc (ΔIsc CFTRinh-172) following cAMP-mediated activation. To assess CFTR maturation, Western blot analysis of whole-cell lysates was performed. Primary airway epithelial cultures were established from nasal brushings of three patients with CF homozygous for F508del. Intracellular uptake of NB1-R10 in these cultures was examined by live-cell microscopy. The effect of NB1-R10 monotherapy on F508del-CFTR function, as well as its synergism with ETI treatment, was assessed using Isc measurements.

Results: Conjugation with R10 enabled dose-dependent intracellular delivery of NB1 in CFBE41o- cells, resulting in enhanced maturation and trafficking of F508del-CFTR to the apical cell membrane, and significantly enhanced F508del-CFTR channel function compared to vehicle-treated control. Furthermore, co-treatment with NB1-R10 and ETI led to a ~3.8-fold increase in F508del-CFTR function compared to ETI alone in CFBE41o- cells. In highly differentiated, patient-derived airway epithelial cultures, treatment with NB1-R10 demonstrated efficient intracellular uptake. Isc measurements revealed significantly increased F508del-CFTR function compared to vehicle (ΔIsc CFTRinh-172 = -2.84 ± 0.19 μA/cm² vs. -1.49 ± 0.07 μA/cm²; $p < 0.001$). ETI therapy alone restored F508del-CFTR function to 55% of normal levels (ΔIsc CFTRinh-172 = -23.01 ± 1.17 μA/cm² vs. -41.52 ± 5.42 μA/cm²). Notably, co-treatment with NB1-R10 and ETI significantly enhanced F508del-CFTR rescue compared to ETI alone (ΔIsc CFTRinh-172 = -36.85 ± 1.54 μA/cm²; $p < 0.001$), achieving ~89% of normal CFTR function.

Conclusions: Our results show that stabilization of F508del-CFTR via efficient delivery of NB1-R10 enhances the functional rescue of CFTR achieved by ETI in airway epithelial cultures from patients with CF. This study highlights the utility of cell-permeable nanobodies for modulating protein function and underscores their therapeutic potential as next generation biopharmaceuticals for intracellular targeting.

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