combination C4+C18. To assess bicarbonate currents, we used a solution containing 125mM $NaHCO_3$ bubbled with 25% CO_2. Isc was measured with equal solutions in both chambers. In these experimental conditions the CFTR generated bicarbonate currents of R344W were nearly identical to those of WT CFTR. They increased above that of WT CFTR when treated with the combination of correctors C4+C18. Given that bicarbonate was the major anion in the solution, the data suggest that R334W supports bicarbonate transport which is almost equal to that of WT CFTR. Taken together our data suggest that the chloride transport through R334W is severely compromised but bicarbonate transport is more like that of WT CFTR. This may explain why these patients have milder pancreatic function. Finally, patients with this mutation could be good candidates for combined corrector therapies. Funded by CFF.

44

CHRONIC LABA EXPOSURE IMPAIRS CFTR ACTIVATION THROUGH cAMP SIGNALING DEFECTS

Beltran Ale, G.J.; Moncivaiz, J.; Ostmann, A.; Strecker, L.; Clancy, J.P.; Brewington, J.J. *Pediatric Pulmonary Medicine, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA*

Objective: Short- and long-acting β2 agonists (SABA/LABA) are traditional therapies in CF for bronchodilation and augmented airway clearance. Subgroup analysis of the TRAFFIC/TRANSPORT data suggests that chronic use of these drugs may reduce lung function benefit from ivacaftor/lumacaftor. Our previous work has shown that chronic SABA exposure limits corrected F508del CFTR activation in vitro. Here we hypothesized that similar effects are produced by LABA exposure, and investigated pharmacokinetics and underlying cause.

Methods: Immortalized CFBE41o- cells transduced with wild-type (wt) or F508del CFTR were grown as monolayers and exposed to study drugs as indicated for 72 hours. F508del cells were corrected with 72-hour exposure to VX-809 (3μM). Cells were then studied under voltage clamp conditions in Ussing chambers, using a set series of drugs to determine CFTR current (stimulation with forskolin/IBMX with an apical low-chloride gradient).

Results: In VX-809-corrected F508del expressing cells exposed continuously to LABA (formoterol, 1μM), a 53% reduction in CFTR currents was noted (6 vs control 13μA/cm^2; p=0.001). Following 72 hours of twice-daily, 1 hour exposures to LABA, VX-809-corrected F508del CFTR currents fell by 46% (7 vs 13μA/cm^2; p=0.006). Intermittent SABA exposure (1 hour albuterol exposure twice daily, 10μM) reduced VX-809-corrected F508del CFTR currents by 39% (8 vs control 13μA/cm^2; p=0.007), while continuous SABA exposure reduced CFTR currents by 37% (8.2μA/cm^2; p=0.0001).

In wtCFTR cells, there was a dose-dependent reduction in CFTR currents following 72 hours of LABA (formoterol, 1μM) exposure, reducing Isc by 77% at 0.1uM (13 vs control 58μA/cm^2; p<0.0001) and by 86% at 1μM (8μA/cm^2; p<0.001). As little as 24 hours of 1μM formoterol reduced CFTR function by 68% (95 vs control 292μA/cm^2; p<0.001), with the effect plateauing after 48 hours of treatment, reducing Isc by 76% (71μA/cm^2; p<0.001). After removal of LABA from the cells, CFTR currents remained reduced by 71% after 24 hours (32 vs control 111μA/cm^2; p=0.04), showed a trend of reduction by 36% after 48 hours off (71μA/cm^2; p=0.20), and returned to normal function at 72 hours off LABA (114μA/cm^2; p=0.93).

Using RNAseq data, few transcriptional changes were produced by 72 hour SABA exposure in wtCFTR or F508del/F508del primary bronchial epithelial cells, suggesting post-transcriptional effects. This, coupled with failed cAMP generation with forskolin stimulation following chronic SABA treatment, implies negative interactions between chronic beta adrenergic receptor stimulation and adenylyl cyclase function.

Conclusions: Chronic, continuous or intermittent exposure to SABA or LABA drugs significantly reduces CFTR activation within 24 hours of drug initiation, and persists 24-48 hours following discontinuation. We speculate that this reduction may be important to CF subjects on modulator therapies, where limited, pharmacologically rescued function may be vulnerable to chronic SABA or LABA treatment. Ongoing understanding of the kinetics of this effect and its application in vivo will aid in determining drug utilization and dosing regimens.

Supported by CFF.

45

TARGETING ERDJ4/DNAJB9, A NOVEL CO-CHAPERONE IN THE ER LUMEN, TO RESCUE MUTANT CFTR

Huang, Y.[1]; Arora, K.[1]; Weaver, T.[2]; Naren, A.[1] *1. Division of Pulmonary Medicine, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA; 2. Division of Pulmonary Biology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA*

Introduction and Objective: Cystic fibrosis is an autosomal, recessive genetic disease affecting multiple organs, including lung, pancreas, and intestine. It is caused by mutation(s) in a gene encoding a chloride channel, cystic fibrosis transmembrane conductance regulator (CFTR). Largely, the disease-related mutations lead to misfolded CFTR which is consequently degraded by endoplasmic reticulum-associated degradation (ERAD). Much of the effort of perturbing the chaperone system involving CFTR biogenesis is attempted from the cytosolic surface. However, there is very little report regarding quality control (QC) for CFTR within the ER lumen.

ER-localized DnaJ 4 (ERdj4), a member of heat shock protein (Hsp) 40 family, is an ER-resident co-chaperone and is upregulated in response to unfolded protein response. A recent study (O'Neal WK, et al. Am J Hum Genet. 2015;96(2):318-28) to correlate gene expression with CF pulmonary phenotype shows that ERdj4 is upregulated in CF patients harboring ΔF508-CFTR (present in ≥90% of CF patients). However, the role of ERdj4 in the biogenesis of CFTR is unknown. This study looks at the potential therapeutic benefit to CF patients by targeting the ERdj4-involved CFTR degradation.

Methods: Interaction between CFTR and ERdj4 was studied using ProtoArray with purified recombinant proteins. Mice with hypomorphic expression of ERdj4 by gene trap (GT) mutagenesis were kindly provided by Dr. Timothy E. Weaver in CCHMC (Fritz JM et al. Mol Biol Cell. 2014;25(4):431-40). In vivo studies were performed by generating ERdj4$^{+/+}$, homozygous for the trapped allele (ERdj4$^{GT/GT}$), CFTR$^{\Delta F508/\Delta F508}$, and ERdj4$^{GT/GT}$/CFTR$^{\Delta F508/\Delta F508}$ mice. Fluid secretion was measured in vivo, using ileal loop experiment. In parallel, ex vivo studies were performed using organoids from the mouse intestine. The effect of ERdj4 in the cell surface expression of CFTR was determined by CFTR functional assays, *e.g.* 6-methoxy-1-(3-sulfonatopropyl) quinolinium (SPQ) assay in HEK293 cells following transient overexpression of ERdj4.

Results: CFTR bound with high affinity to ERdj4 in ProtoArray experiment. ERdj4$^{GT/GT}$ mice, compared to wild-type control, showed enhanced fluid secretion, both in vivo ileal loop experiment and ex vivo intestinal organoid studies. ERdj4 $^{GT/GT}$/CFTR$^{\Delta F508/\Delta F508}$ mice had higher organoid fluid secretion compared to CFTR$^{\Delta F508/\Delta F508}$. Overexpression of ERdj4 in cells expressing CFTR resulted in reduced CFTR function, reflected by the decreased SPQ fluorescent signal compared to control.

Conclusions: ERdj4 shows direct interaction with CFTR and participates in QC of CFTR synthesis within the ER. Manipulating this chaperone system (ERdj4) in the ER lumen may enhance cell surface functional expression of CFTR. Targeting ERdj4 may lead to development of new therapeutic strategies.

Acknowledgements: This study is supported by CFF Postdoctoral Fellowship HUANG17F0.

46

DECIPHERING THE MODE OF ACTION OF CLINICALLY RELEVANT NEXT GENERATION C2 CORRECTOR COMPOUNDS GLPG2737 AND GLPG3221

Peters, F.[1]; Sahasrabudhe, P.[1]; De Wilde, G.[2]; Kleizen, B.[1]; Conrath, K.[2]; Braakman, I.[1] *1. Cellular Protein Chemistry, Utrecht University, Utrecht, Netherlands; 2. Galapagos N.V., Mechelen, Belgium*

The current therapeutic strategy to repair cystic fibrosis-causing defects in the chloride channel CFTR is to develop novel and better correctors (to improve folding) and potentiators (to improve function). Galapagos-AbbVie identified C2 correctors by high-throughput compound screening and Med Chem optimization for cell surface rescue of F508del-CFTR. These C2 correctors are acting synergistically with a type I corrector such as ABBV/GLPG2222. Two C2 correctors, ABBV/GLPG2737 and ABBV/

GLPG3221 were optimized for drug like properties and are in clinical and pre-clinical evaluation, respectively.

From both the functional halide efflux assays and pulse chase analysis we showed that the rescue efficiency of F508del-CFTR after combination treatment (C1 + C2) is markedly higher (≥50% of wild-type levels) than the sum of C1 and C2 correction. These strong synergistic effects show not only that C1 and C2 have a different mode of action, but also highlight the benefit of the triple-combination treatment with addition of a potentiator.

To investigate how, when and where these C2 correctors act on CFTR we use radiolabeling approaches in combination with protease susceptibility assays. We first evaluated C1 corrector ABBV/GLPG2222 using in vitro translation and translocation assays in the presence of semi-intact HEK293 cells as source for endoplasmic reticulum (ER) membranes. We found that ABBV/GLPG2222, but not the C2 correctors, acted on transmembrane domain 1 (TMD1) in an identical fashion as lumacaftor by promoting its cytoplasmic loop packing important for domain folding.

Varying the time of drug addition in pulse chase experiments showed that, like C1 corrector, both C2 correctors reached maximal rescue efficiency when present during, and shortly after the 15-minute pulse labelling. The C2 correctors acted additively with all F508del suppressors (I539T, G550E and R1070W) and did not restore nucleotide binding domain 1 (NBD1) folding in the F508del-CFTR background. Although we did not identify yet where the C2 correctors act, these compounds restored trafficking of the NBD2-less F508del-CFTR (F508del-1219X) construct very well.

Our results show that the C2 correctors promote the earliest folding events of the ER-export competent CFTR molecule lacking NBD2, ruling out all possible NBD2 inter-domain assembly events (TMD1/NBD2; NBD1/NBD2; TMD2/NBD2) as target candidate. The triple-combination treatment that includes these C2 correctors significantly raises the F508del-CFTR rescue ceiling, with the aim to reach sufficient clinical benefit for most CF patients in the near future.

48

SYNERGISTIC RESCUE OF ΔF508 AND CFTR2 MUTATION FUNCTIONAL EXPRESSION DEFECT BY STRUCTURE-GUIDED CORRECTOR COMBINATION

Veit, G.[2]; Avramescu, R.G.[2]; Xu, H.[2]; Bagdany, M.[2]; Beitel, L.[2]; Roldan, A.[2]; Lay, C.[1]; Li, W.[1]; Morin, K.[1]; Gao, S.[1]; Mak, A.[1]; Ainscow, E.[1]; Orth, T.[1]; McNamara, P.[1]; Frenkiel, S.[2]; Matouk, E.[2]; Barnes, W.[1]; Lukacs, G.L.[2] *1. Genomic Institute of the Novartis Research Foundation, San Diego, CA, USA; 2. McGill University, Montreal, QC, Canada*

The most common cystic fibrosis mutation, ΔF508 in nucleotide binding domain 1 (NBD1), impairs CFTR-coupled domain folding, plasma membrane (PM) expression, function, and stability. Robust ΔF508-CFTR correction is achieved by stabilization of NBD1, the interfaces between NBD1 and membrane-spanning domains (MSDs), as well as NBD2, the former two representing primary conformational defects, established by using combination of genetic and pharmacological means (1). Thus, a rationally designed, structure-guided corrector strategy may require the combination of class I correctors supporting the NBD1-MSD1 and NBD1-MSD2 interface formation, class II correctors targeting NBD2, and class III correctors stabilizing the NBD1 domain (1).

VX-809 (lumacaftor), the only approved corrector, exhibits a class I mechanism and in combination with the gating potentiator VX-770 (ivacaftor) provides only modest clinical benefit to patients carrying two copies of the ΔF508 mutation (2).

Here we report the identification of compounds for all three corrector classes in a screen of ~600,000 small molecules by monitoring the PM expression of the HRP-tagged ΔF508 in CFBE41o- (CFBE) epithelia. Compounds that increased ΔF508 PM densities both in the presence and absence of VX-809 contained class III correctors, compounds that required VX-809 included class II correctors, and compounds exhibiting redundancy with VX-809 encompassed class I correctors. The mechanisms of action (MOAs) of correctors were determined by domain-interrogation and domain-specific binding assays, competition with reference compounds, and ΔNBD2-CFTR PM density measurements. While class I-III correctors alone displayed only modest correction, combination of correctors from all three classes synergistically increased the Δ508 PM density and function in CFBE by up to ~9 fold in comparison to VX-809 alone, augmented the mutant endoplasmic reticulum maturation and the abundance of the complex-glycosylated form, promoted the peripheral stability, and largely normalized the single channel function of ΔF508. These results correlated well with CFTR gain-of-function in human bronchial epithelia and human nasal epithelia from $CFTR^{\Delta F508/\Delta F508}$ patients. The effect of corrector combinations was determined on forskolin plus genistein activated and Inh_{172}-sensitive short circuit current and reached ~50% of the corresponding wild-type currents. Moreover, ongoing experiments suggest that corrector combination can restore the PM density and function of several CFTR2 (www.cftr2.org) processing mutants in CFBE cells.

This study provides proof of principle for synergy screening to identify correctors with distinct MOAs, which, when used in structure-guided combinations, achieve therapeutically relevant correction levels of ΔF508 and other processing mutants.

References:

1. Okiyoneda T, et al. Nat Chem Biol 2013;9(7):444-54.
2. Wainwright CE, et al. N Engl J Med 2015;373(3):220-31.

49

THE EGFR/ERK AXIS REGULATES EXPRESSION OF CFTR UPON EXPOSURE TO THE ACTIVE SUBSTANCE OF CANNABIS, Δ9-TETRAHYDROCANNABINOL (THC)

Chang, S.[1]; Wellmerling, J.[1]; Peeples, M.[2,3]; Boyaka, P.[1]; Cormet-Boyaka, E.[1] *1. Veterinary Biosciences, The Ohio State University, Columbus, OH, USA; 2. Center for Vaccines and Immunity, Nationwide Children's Hospital, Columbus, OH, USA; 3. Department of Pediatrics, The Ohio State University, Columbus, OH, USA*

Cannabis smoking is on the rise in the United States as the legalization of recreational and medicinal marijuana is becoming more common. The beneficial effects of cannabis are controversial and the impact of cannabis smoking on lung homeostasis is not well-understood, particularly due to the various amounts of the psychoactive substance Δ9-tetrahydrocannabinol (THC) present in marijuana. Even though the effect of marijuana smoking on obstructive lung disease varies among studies, it is recognized that marijuana smoking is associated with increased cough, sputum production, and chronic bronchitis. We and others have shown that patients with chronic bronchitis due to long-term tobacco smoking have reduced expression of the cystic fibrosis transmembrane conductance regulator (CFTR), an ion channel that regulates fluid homeostasis in the lung, suggesting that tobacco smoking could lead to "acquired" chronic bronchitis. In this study, we investigated the effect of THC on the expression and function of CFTR. Our data show that THC decreases CFTR protein expression in a dose-dependent manner in human bronchial epithelial cells. This decrease was associated with reduced CFTR short-circuit currents in primary human airway cells. Since we recently demonstrated that cigarette smoke promotes degradation of CFTR via activation of the MEK/ERK MAPK pathway, we wondered whether this latter signaling pathway was responsible for the THC-mediated decrease of CFTR. Inhibition of MEK/ERK using specific inhibitors prevented THC-induced decrease of CFTR protein. In addition, we found that inhibition of the EGF receptor blocked activation of ERK after exposure to THC, and prevented loss of CFTR protein as observed by immunoblotting. Taken together, our results show that THC negatively regulates CFTR and identify the EGFR/ERK axis as a key regulator of CFTR in the lungs.

50

IDENTIFICATION OF A RARE CFTR MUTATION THAT RESPONDS TO VX-809

Cotton, C.[1]; Wilson, M.[1]; Thomas, P.J.[2]; Chmiel, J.[1,3] *1. Case Western Reserve University, Cleveland, OH, USA; 2. Physiology, UT-Southwestern Medical School, Dallas, TX, USA; 3. Pediatrics, Rainbow Babies & Children's Hospital, Cleveland, OH, USA*

Primary human airway epithelial cell cultures maintained at air/liquid interface (ALI) are an important model system for preclinical testing of CFTR modulators. Human bronchial epithelial (HBE) cells obtained from transplanted CF lungs were used extensively in the development of VX-770 (ivacaftor) and VX-809 (lumacaftor) as modulators to improve