WS1.1 The effect of ivacaftor, a CFTR potentiator, in patients with cystic fibrosis and a non-*G551D-CFTR* gating mutation, the KONNECTION study

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Background: Ivacaftor is a CFTR potentiator approved for use in CF patients ⩾6 years of age with the *G551D* gating mutation. This study evaluated the safety and efficacy of ivacaftor treatment in patients with CF who were ⩾6 years old, had a non-*G551D* Class III gating mutation on at least one allele, and an FEV_1 >40% of predicted at screening.
Methods: This two-part, Phase 3 study consisted of a randomized, placebo-controlled, double-blind, 8-week crossover period and a 16-week open-label extension period (OLE). Thirty-nine patients were screened and randomized to one of two treatment sequences including 8 weeks of ivacaftor treatment and 8 weeks of placebo. The primary outcome measure was the absolute change from baseline in % predicted FEV_1 through 8 (Part 1) and 24 weeks (including the 16-week OLE, Part 2) of treatment. Secondary outcome measures included absolute change from baseline in BMI at 8 and 24 weeks, and absolute change from baseline in sweat chloride and CFQ-R through 8 and 24 weeks.
Results: In Part 1, in the intention-to-treat full analysis set, patients receiving ivacaftor for 8 weeks had significant improvements in % predicted FEV_1, BMI, and sweat chloride (all $P < 0.0001$) and pooled CFQ-R domain score ($P = 0.0004$), compared with placebo. Results from Part 2 will be available for presentation.
Conclusions: Eight weeks of ivacaftor treatment provided clinically relevant and statistically significant improvements compared with placebo in lung function, BMI, sweat chloride, and CFQ-R in patients with CF and a non-*G551D* gating mutation, consistent with the *G551D* gating trials. Data from Part 2 of this study will address the durability of these results from Part 1.

WS1.2 Vardenafil promotes relocalization of F508del-CFTR in human and mouse airways

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Objectives: Vardenafil, a clinically approved cGMP-dependent phosphodiesterase type 5 inhibitor (PDE5i), normalizes defective F508del-CFTR chloride transport across the nasal mucosa of mice carrying the F508del mutation (CF). This work aimed at evaluating the influence of vardenafil on CFTR expression and localization in mouse lungs and in human bronchial epithelial cells in culture.
Methods: CFTR localization was studied by immunohistostaining and evaluated by a normalized ratio of specific fluorescence (CFTR antibody clone 24-1) between the apical and the subapical compartments of cells. Incubation of slides with Pontamine Sky Blue was performed during 10 minutes before immunostaining to overcome lung tissue autofluorescence.
Results: Immunohistostaining studies showed reduced CFTR expression in apical compartments of CF airways: in untreated conditions, the apical/subapical fluorescence ratio was lower in CF (1.48±0.09) than in WT tissues (2.24±0.12; $p < 0.0001$). In CF tissues, vardenafil treatment increased both the ratio of fluorescence and the peak intensity of the CFTR signal to WT values.
Conclusion: These data show that vardenafil acts as a CFTR corrector by promoting CFTR relocalization of the protein towards and into the apical compartment where the wild-type protein is mainly expressed. This work provides compelling support for studying cGMP pathway as therapeutic strategies in CF pharmacotherapy.

WS1.3 Enhanced correction of F508del CFTR using drug-like small molecules in combination with correctors and potentiators

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Objectives: The most common CF causative mutation in CFTR is the deletion of phenylalanine 508 (F508del), resulting in a rapidly degraded protein that fails to progress out of the ER and traffic to the cell surface. Multiple strategies to correct F508del mutant CFTR with small molecules have been employed. For example, pharmacological chaperones directly bind to and improve the mutant protein's folding stability, while protein homeostasis regulators modulate the cellular folding environment to promote folding, trafficking, and maturation of the mutant protein. Proteostasis Therapeutics, Inc. (PTI) is pursuing regulators of the protein homeostasis network (PN) that improve the folding, trafficking, and function of F508del CFTR.
Methods: We performed a high-throughput screen of small molecules that modulate PN pathways using our Multiplex Gene Expression platform. Hit compounds were tested for correction of F508del CFTR using electrophysiology-based assays in primary human bronchial epithelial lung cells.
Results: Multiple active chemical series were found. PTI Corrector Series B showed particularly robust activity and has undergone further characterization. Series B demonstrates substantial correction in combination with a potentiator. Combination of Series B with known correctors plus a potentiator more than doubles the F508del CFTR functional rescue of these agents.
Conclusion: Series B has good drug-like physicochemical and ADME properties, providing a strong foundation for our drug discovery efforts to develop clinically efficacious CF therapeutics.
Funded in part by a Therapeutics Development Award from Cystic Fibrosis Foundation Therapeutics, Inc.

WS1.4 A rAAV2/5 based gene therapy model for cystic fibrosis airway disease

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Gene therapy (GT) can treat CF in a curative, mutation independent manner. In this study, we aim to develop a GT-based mouse model for CF using rAAV2/5 viral vectors, which efficiently transduce airway epithelium. As transduction of dividing airway epithelium with a non-integrating vector requires repeat administration for long-term correction, we hypothesized that immune system immaturity during perinatal GT would prevent an immune response against the vector, allowing repeat administration.
We administered rAAV2/5-fLUC (luciferase) to the fetal and neonatal mouse airways which resulted in a comparable fLUC signal in the lungs. However, fLUC activity decreased 5-fold at 3 mo. As only low to absent neutralizing antibodies (nAb) against the vector were present, this allowed readministration at 3 mo resulting in stable gene expression till 6 mo. To replace reporter genes by *CFTR*, we had to overcome the size limit of rAAV (~5 kb). Therefore, we functionally validated a truncated CFTR with a deletion in the R-domain (CFTRΔR) described by Ostedgaard *et al*. Upon forskolin and genistein stimulation, CFTRΔR showed a $^{125}I^-$ efflux comparable to WT CFTR in stable HeLa cell lines. After in house production, vector titers were obtained comparable to control vectors. Additionally, rAAV2/5-CFTRΔR was functional on the mRNA level measured by qPCR in transduced 293T cells.
In conclusion, we obtained long-term gene expression in the mouse airways after perinatal rAAV2/5 administration and readministration using reporter genes. Currently, we are investigating perinatal rAAV2/5 administration in CF mice to assess CFTR expression and function by nasal potential difference measurements.