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 FEV₁ ≥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 FEV₁ 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 FEV₁, BMI, and sweat chloride (all P < 0.0001) and pooled CFQ-R domain scores (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.

Oral Presentations

Workshop 1: Fixing basic defects – New therapies

### WS1.1

#### 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.

### WS1.2

#### 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-ILUC (luciferase) to the fetal and neonatal mouse airways which resulted in a comparable ILUC signal in the lungs. However, ILUC activity decreased 5-fold at 3mo. As only low to absent neutralizing antibodies (nAb) against the vector were present, this allowed readministration at 3mo resulting in stable gene expression till 6mo. To replace reporter genes by CFTR, we had to overcome the size limit of rAAV (<5kb). Therefore, we functionally validated a truncated CFTR with a deletion in the R-domain (CFTRAR) described by Ostedgaard et al. Upon forskolin and genistein stimulation, CFTRAR showed a 121-fold more efficient compared to WT CFTR in stable HeLa cell lines. After in house production, vector titers were obtained comparable to control vectors.

Additionally, rAAV2/5-CFTRAR 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.