Effects of Lumacaftor-Ivacaftor on Lung Clearance Index, Magnetic Resonance Imaging and Airway Microbiome in Phe508del Homozygous Patients with Cystic Fibrosis

Simon Y. Graeber[1,2,3,4,5,6]*, Sébastien Boutin[6,7]*, Mark O. Wielpütz[6,8], Cornelia Joachim[5], Dario L. Frey[4,6], Sabine Wege[6,9], Olaf Sommerburg[5,6], Hans-Ulrich Kauczor[6,8], Mirjam Stahl[1,3,4,5,6], Alexander H. Dalpke[6,7,10], Marcus A. Mall[1,2,3,4,5,6]

[1]Department of Pediatric Pulmonology, Immunology and Critical Care Medicine and Cystic Fibrosis Center, Charité - Universitätsmedizin Berlin, Berlin, Germany
[2]Berlin Institute of Health (BIH), Berlin, Germany
[3]German Centre for Lung Research (DZL), associated partner site, Berlin, Germany
[4]Department of Translational Pulmonology, University of Heidelberg, Heidelberg, Germany
[5]Division of Pediatric Pulmonology & Allergy and Cystic Fibrosis Center, Department of Pediatrics, University of Heidelberg, Heidelberg, Germany
[6]Translational Lung Research Center Heidelberg (TLRC), German Center for Lung Research (DZL), University of Heidelberg, Heidelberg, Germany
[7]Department of Infectious Diseases, Medical Microbiology and Hygiene, University Hospital Heidelberg, Germany
[8]Department of Diagnostic and Interventional Radiology, University of Heidelberg, Heidelberg, Germany
[9]Department of Pneumology and Respiratory Critical Care Medicine, Thoraxklinik, University Hospital Heidelberg, Heidelberg Germany
[10]Institute of Medical Microbiology and Hygiene, Medical Faculty, Technische Universität Dresden, Dresden, Germany
* S.Y.G. and S.B. contributed equally to this work.

Correspondence should be addressed to:
Simon Y. Graeber, M.D., Department of Pediatric Pulmonology, Immunology and Critical Care Medicine, Charité - Universitätsmedizin Berlin and Berlin Institute of Health (BIH), Augustenburger Platz 1, 13353 Berlin, Germany; Phone: +49 (30) 450616434, Fax: +49 (30) 450566931, E-Mail: simon.graeber@charite.de
Marcus A. Mall, M.D., Department of Pediatric Pulmonology, Immunology and Critical Care Medicine, Charité - Universitätsmedizin Berlin and Berlin Institute of Health (BIH), Augustenburger Platz 1, 13353 Berlin, Germany; Phone: +49 (30) 450566182, Fax: +49 (30) 450566931, E-Mail: marcus.mall@charite.de

Author Contributions: Conception and design of the study: S.Y.G., S.B., M.O.W. A.H.D., M.A.M.; acquisition, analysis and interpretation of data: S.Y.G., S.B., M.O.W. C.J. D.L.F., S.W., O.S., H.U.K., M.S., A.H.D., M.A.M.; drafting the article or revising it critically for important intellectual content: S.Y.G., S.B., M.O.W. C.J. D.L.F., S.W., O.S., H.U.K., M.S., A.H.D., M.A.M..

Grant Support: This work was supported in part by grants from the German Federal Ministry of Education and Research (82DZLE12A1 and 82DZL004A1) and the Einstein Foundation Berlin (EP-2017-393 to M.A.M). S.Y.G. is participant in the BIH-Charité Clinician Scientist Program funded by the Charité – Universitätsmedizin Berlin and the Berlin Institute of Health.

ANNALSATS Articles in Press. Published February 18, 2021 as 10.1513/AnnalsATS.202008-1054OC

Running Head: Lumacaftor-ivacaftor effects on CF lung disease

Subject Category: 9.17 Cystic Fibrosis: Translational & Clinical Studies

Key Words: cystic fibrosis, lumacaftor-ivacaftor, LCI, MRI, airway microbiome

Word Count: 3972

This article has an online supplement, which is accessible from this issue's table of contents online at www.atsjournals.org

ANNALSATS Articles in Press. Published February 18, 2021 as 10.1513/AnnalsATS.202008-1054OC

Abstract

Rationale: Previous studies showed that lumacaftor-ivacaftor therapy results in partial rescue of cystic fibrosis transmembrane conductance regulator (CFTR) activity and moderate improvement of spirometry in Phe508del homozygous patients with cystic fibrosis (CF). However, the effects of lumacaftor-ivacaftor on lung clearance index (LCI), lung morphology and perfusion detected by chest magnetic resonance imaging (MRI), and effects on the airway microbiome and inflammation remain unknown.

Objectives: To investigate the effects of lumacaftor-ivacaftor on LCI, lung MRI scores, and airway microbiome and inflammation.

Methods: In this prospective observational study we assessed clinical outcomes including spirometry and body mass index, LCI, lung MRI scores, sputum microbiome and pro-inflammatory cytokines in 30 Phe508del homozygous patients with CF 12 years and older before and 8-16 weeks after initiation of lumacaftor-ivacaftor therapy.

Results: Lumacaftor-ivacaftor had no effects on FEV_1 % predicted (1.7%, 95% confidence interval (CI) -1.0 to 4.3%; $P = 0.211$), but improved LCI (-1.6, 95% CI -2.6 to -0.5; $P < 0.01$) and MRI morphology (-1.3, 95% CI -2.3 to -0.3; $P < 0.05$) and perfusion score (-1.2, 95% CI -2.3 to -0.2; $P < 0.05$) in our study cohort. Further, lumacaftor-ivacaftor decreased the total bacterial load (-1.8, 95% CI -3.3 to -0.34; $P < 0.05$) and increased the Shannon diversity of the airway microbiome (0.4, 95% CI 0.1 to 0.8; $P < 0.05$), and reduced IL-1β levels (median change -324.2 pg/ml, 95% CI -938.7 to 290.4 pg/ml; $P < 0.05$) in sputum of Phe508del homozygous patients.

Conclusions: This study shows that lumacaftor-ivacaftor has beneficial effects on lung ventilation, morphology and perfusion, as well as on the airway microbiome and inflammation

ANNALSATS Articles in Press. Published February 18, 2021 as 10.1513/AnnalsATS.202008-1054OC

in Phe508del homozygous patients. Our results suggest that LCI and MRI may be more sensitive than FEV_1 % predicted to detect response to CFTR modulator therapy in patients with chronic CF lung disease.

Clinical trial registered with ClinicalTrials.gov (NCT02807415)

ANNALSATS Articles in Press. Published February 18, 2021 as 10.1513/AnnalsATS.202008-1054OC

The combination of the CFTR corrector lumacaftor with the potentiator ivacaftor was the first CFTR modulator drug approved for the treatment of patients with CF homozygous for the common Phe508del mutation. In clinical trials, lumacaftor-ivacaftor showed a moderate improvement in mean forced expiratory volume in one second (FEV_1 % predicted) with substantial variability between individual patients, improvement in body mass index (BMI) and a reduction of pulmonary exacerbations (1-6). We previously demonstrated that lumacaftor-ivacaftor leads to partial rescue of CFTR activity to levels of ~10 to 20 % of normal CFTR function, which was also observed in patients that did not show short-term improvement in FEV1 % predicted after initiation of lumacaftor-ivacaftor therapy (7-9). These findings suggest that FEV_1 % predicted has potential limitations as outcome measure of response to CFTR modulators, which may be related to fixed airflow obstruction caused by irreversible structural lung damage that may render spirometry insensitive to detect therapeutic benefits associated with the level of CFTR function induced by lumacaftor-ivacaftor.

In this context, multiple breath washout (MBW) as an outcome measure of ventilation homogeneity and cross-sectional imaging by chest computed tomography (CT) or magnetic resonance imaging (MRI) have emerged as promising alternative outcome measures to detect response to therapeutic interventions. First, the lung clearance index (LCI) derived from MBW has been shown to be more sensitive than FEV_1 % predicted to detect response to therapeutic interventions in patients with preserved spirometry including lumacaftor-ivacaftor therapy in children and adolescents with CF (10-19). Further, chest CT and hyperpolarized helium-3 MRI detected improvements in CF patients with a Gly551Asp mutation after starting CFTR modulator therapy with ivacaftor (20-22). We previously demonstrated that conventional

ANNALSATS Articles in Press. Published February 18, 2021 as 10.1513/AnnalsATS.202008-1054OC

proton MRI as a widely available radiation-free imaging technique is sensitive to detect abnormalities in lung morphology and perfusion that correlated with ventilation inhomogeneity detected by MBW in children and adolescents with CF (14, 23-26). So far, MBW has rarely been used for the assessment of lung function in adult CF patients with chronic lung disease and the effect of lumacaftor-ivacaftor therapy on LCI in patients 12 years and older with impaired spirometry remains unknown. Similar, the effects of lumacaftor-ivacaftor on abnormalities in lung structure and morphology in Phe508del homozygous patients with CF have not been studied.

Emerging evidence suggests that CF lung disease is characterized by polymicrobial airways infection and that changes in airway microbiota may play an important role in disease severity and progression in patients with CF (26). It was found that the emergence of a pathogen-dominated airway microbiome is associated with airway inflammation and the onset of structural lung disease from early childhood (27) and that progressive loss of diversity of bacterial species in sputum is associated with decreased lung function in older CF patients with chronic lung disease (28-31). Therefore, the effects of CFTR modulators on the structure and diversity of the airway microbiome, as well as airway inflammation are of great interest. While previous studies in CF patients with a Gly551Asp mutation treated with ivacaftor showed mixed results (21, 22, 32, 33), the effects of lumacaftor-ivacaftor on sputum microbiome and inflammation markers in Phe508del homozygous patients with CF have not been determined.

The aim of our study was to determine the effects of lumacaftor-ivacaftor on novel endpoints of lung ventilation, lung structure and perfusion as well as airway infection and inflammation in patients with CF homozygous for the Phe508del mutation 12 years and older.

ANNALSATS Articles in Press. Published February 18, 2021 as 10.1513/AnnalsATS.202008-1054OC

To achieve these goals, we initiated a prospective observational study in Phe508del homozygous patients and assessed the effects on lung function with spirometry and MBW, on lung structure and perfusion by lung MRI, and on the airway microbiome and inflammation markers in sputum at baseline and after 8 to 16 weeks of initiation of lumacaftor-ivacaftor therapy in a real-world post-approval setting. Some of the results of these studies have been previously reported in the form of abstracts (34-36).

Methods

Study Design and Participants

This prospective observational post-approval study was approved by the ethics committee of the University of Heidelberg (S-370/2011) and written informed consent was obtained from all patients, their parents or legal guardians. Patients were eligible to participate if they were at least 12 years old, homozygous for the Phe508del *CFTR* mutation, had no prior exposure to lumacaftor-ivacaftor. 30 patients were included (Table 1) and anthropometry, spirometry, sweat chloride concentrations, MBW, MRI and sputum analysis were performed at baseline and 8 to 16 weeks after initiation of therapy with the approved dose of lumacaftor 400 mg in combination with ivacaftor 250 mg every 12 h. In this real-world study in the clinical setting, MRI studies were limited to 14 patients, in part due to the limited availability of MRI scan time at the scheduled baseline and follow-up visits. Additional details are provided in the online supplement.

ANNALSATS Articles in Press. Published February 18, 2021 as 10.1513/AnnalsATS.202008-1054OC

Multiple Breath Washout (MBW)

MBW testing was performed with the Exhalyzer D system / spiroware 3 (Eco Medics, Duernten, Switzerland) and 100% oxygen was used to wash out resident nitrogen from the lungs using a mouthpiece as interface (14, 37-39) as detailed in the online supplement.

Morpho-functional Chest Magnetic Resonance Imaging (MRI)

T1-weighted sequences before and after intravenous contrast, T2-weighted sequences, and first-pass perfusion imaging were acquired using a clinical 1.5T MRI scanner (Magnetom Avanto, Siemens, Erlangen, Germany) and images were assessed for abnormalities in lung morphology and perfusion using a dedicated morpho-functional MRI score (14, 23-25, 40) as detailed in the online supplement.

Sputum Microbiome Analysis

DNA extraction and next generation sequencing (NGS) of v4 region of the 16S rRNA gene were performed as previously described (30, 41). Additional details are provided in the online supplement.

Sputum Cytokine Measurements

Concentrations of interleukin-1β (IL-1β), interleukin-6 (IL-6), interleukin-8 (IL-8) and tumor necrosis factor alpha (TNF-α) and free neutrophil elastase (NE) activity were determined in cell-free sputum supernatant. Cytokines were quantified using commercially available cytometric bead array (CBA) kits (BD Biosciences, San Jose, California, USA) according to the

ANNALSATS Articles in Press. Published February 18, 2021 as 10.1513/AnnalsATS.202008-1054OC

manufacturer's instructions. Additional details for measurement of NE activity are provided in the online supplement.

Statistical Analysis

Data were analyzed with R 3.4.4 (42) and the packages microbiome (43) and phyloseq (44). Parametric data are presented as mean and standard deviation and paired comparisons were performed with a paired Student *t* test. To exclude the impact of confounders on changes in lung function (FEV1 % predicted and LCI) or alpha-diversity, a two-way ANOVA was performed using age, FEV_1 % predicted at baseline, exacerbations and antibiotic therapy as co-factors showing no significant impact of those factors. Non-parametric data are presented as median and interquartile range (IQR) and were tested by Wilcoxon signed-rank test. The amplicon sequence variants (ASV) table was used to calculate descriptive indices for alpha-diversity (Shannon index), richness (number of ASV observed) and dominance (relative abundance of the most dominant ASV). Sample size calculation was performed with a paired t-test with a power of 0.8 and an α-level of 0.05. $P < 0.05$ was accepted to indicate statistical significance. Additional details are provided in the online supplement.

Results

Between December 2015 and October 2017, 30 patients with CF homozygous for the Phe508del CFTR mutation aged 12 years and older were enrolled to assess anthropometry, spirometry as well as MBW, chest MRI, sputum microbiome and pro-inflammatory cytokines

ANNALSATS Articles in Press. Published February 18, 2021 as 10.1513/AnnalsATS.202008-1054OC

before and during treatment with lumacaftor-ivacaftor therapy. All patients had successful sweat chloride and spirometry measurements at both time points. 28 patients successfully performed paired MBW measurements, 14 patients underwent paired lung MRI studies and 14 patients provided paired sputum samples at baseline and 8-16 weeks after initiation of lumacaftor-ivacaftor. Demographics and clinical characteristics at baseline and at follow-up are summarized in Table 1. The mean patient age was 20.2 ± 8.1 years and 47% were female (Table 1). As observed in previous studies (4, 7, 45), sweat chloride concentration was significantly reduced by lumacaftor-ivacaftor therapy from 87.6 ± 9.5 mmol/l to 71.3 ± 13.9 mmol/l (mean change -16.0 ± 12.6 mmol/l, $P < 0.001$; Figure 1A). Consistent with results from previous clinical trials (2, 3), BMI increased by 0.3 ± 0.7 kg/m² ($P < 0.05$) under lumacaftor-ivacaftor therapy (Table 1). Mean FEV_1 % predicted was 65.4 ± 19.3 % predicted at baseline and increased by 1.7 ± 7.4 % after starting lumacaftor-ivacaftor therapy, however, this change did not reach statistical significance with the number of patients included this study ($P = 0.211$; Figure 1B).

To determine the effects of lumacaftor-ivacaftor on ventilation homogeneity, we performed MBW measurements at baseline and after initiation of lumacaftor-ivacaftor. Mean LCI was 16.6 ± 6.8 at baseline (upper limit of normal from healthy controls: 8.5) and was significantly improved to 15.1 ± 6.3 (mean absolute change of -1.6 ± 2.9, $P < 0.01$; Figure 1C) under lumacaftor-ivacaftor. This absolute change in LCI corresponded to a relative improvement of 9.4 % compared to baseline.

We further investigated the effects of lumacaftor-ivacaftor therapy on lung morphology and perfusion detected by MRI. Characteristics of patients participating in the MRI substudy were similar compared to the core study (Table E1 in the online supplement). At baseline, MRI

ANNALSATS Articles in Press. Published February 18, 2021 as 10.1513/AnnalsATS.202008-1054OC

showed morphological abnormalities such as bronchiectasis/wall thickening, mucus plugging and pleural reactions, as well as perfusion abnormalities (Figure 2A) resulting in a mean MRI morphology score of 14.7 ± 7.4 (Figure 2B), MRI perfusion score of 6.6 ± 2.6 (Figure 2C) and MRI global score of 21.3 ± 9.6 (Figure 2D). Under treatment with lumacaftor-ivacaftor the MRI morphology score was significantly reduced to 13.4 ± 7.3 (mean change -1.3 ± 1.9, $P < 0.05$; Figure 2B). This change was mainly determined by improvement in the pleural reaction subscore, whereas the other MRI morphology subscores did not change under lumacaftor-ivacaftor (Table E2 in the online supplement). In addition, the MRI perfusion score was improved to 5.4 ± 3.5 (mean change -1.2 ± 2.0, $P < 0.05$; Figure 2C) resulting in a significant improvement in the MRI global score to 18.8 ± 10.5 (mean change -2.5 ± 3.2, $P < 0.05$) under lumacaftor-ivacaftor therapy (Figure 2D).

Next, we investigated the effects of improvement of CFTR function by lumacaftor-ivacaftor on the airway microbiome in a subset of patients with spontaneously expectorated sputum at both time points. These patients were slightly older and tended to have more severe airflow limitation compared to the core study (Table E3 in the online supplement). At baseline, *Pseudomonas aeruginosa* was detected in five patients (Figure 3A). Other common bacteria were *Staphylococcus aureus*, and anaerobes such as *Neisseria*, *Veillonella* and *Prevotella species*. The abundance of single genera did not change under lumacaftor-ivacaftor (Figure 3A). However, the total bacterial load was significantly reduced under treatment with lumacaftor-ivacaftor (mean change -1.8 ± 2.6, $P < 0.05$; Figure 3B). Further, the Shannon diversity of the microbiome was significantly increased under lumacaftor-ivacaftor (mean change 0.4 ± 0.6, $P < 0.05$; Figure 3B). As one factor of the Shannon diversity, the richness of the microbiome showed

ANNALSATS Articles in Press. Published February 18, 2021 as 10.1513/AnnalsATS.202008-1054OC

a trend toward increase under lumacaftor-ivacaftor therapy (mean change 34.1 ± 60.1, *P* = 0.053), whereas dominance, defined as the relative abundance of the most dominant species, remained unchanged (mean change -0.08 ± 0.19, *P* = 0.150; Figure 3C and 3D).

To determine effects of lumacaftor-ivacaftor on airway inflammation, we analyzed levels of pro-inflammatory cytokines and free NE activity that were previously implicated in CF lung disease severity and progression in sputum samples (46-50). At baseline, we found high levels of IL-1β, IL-6, IL-8, TNF-α and free NE activity in our study cohort of adolescent and adult Phe508del homozygous CF patients (Figure 4A-E). After initiation of lumacaftor-ivacaftor mean IL-1β levels were significantly reduced to about 25% of baseline values (median change -324.2 pg/ml, IQR -1273.6 – 28.6 pg/ml, *P* < 0.05; Figure 4A). Levels of IL-6 (median change 99.8 fg/ml, IQR -555.7 - 999.3, *P* = 0.808), IL-8 (median change -20.1 pg/ml, IQR -3840.4 - 3889.5, *P* = 0.855),TNF-α (median change -286.4 fg/ml, IQR -2742.5 - 13871.4, *P* = 0.952) and free NE activity (median change 0.0 µg/ml, IQR -13.7–5.6, p=0.470) remained unchanged after starting lumacaftor-ivacaftor therapy (Figure 4B-E).

Discussion

Lumacaftor-ivacaftor was the first approved CFTR modulator therapy for Phe508del homozygous patients with CF and was shown to improve CFTR function in nasal and intestinal epithelia to a level of 10-20% of normal CFTR activity in this group of patients (7). This prospective observational study is the first to examine the effect of this level of improvement of mutant CFTR function achieved by lumacaftor-ivacaftor on lung ventilation determined by

ANNALSATS Articles in Press. Published February 18, 2021 as 10.1513/AnnalsATS.202008-1054OC

MBW, abnormalities in lung morphology and perfusion determined by chest MRI, and airway microbiome and inflammation constituting key drivers of lung disease progression in adolescent and adult patients with CF. First, we showed that initiation of lumacaftor-ivacaftor therapy was associated with improvement in LCI. Second, we found that lumacaftor-ivacaftor improved changes in lung morphology and perfusion detected by MRI. Third, changes in the airway microbiome and reduced sputum inflammation were observed after initiation of lumacaftor-ivacaftor therapy. These results provide important initial insights into the relationship between the level of functional correction of mutant CFTR and the impact on airway mucus obstruction, polymicrobial infection and inflammation in adolescent and adult CF patients with chronic lung disease.

In previous studies, LCI was shown to detect ventilation inhomogeneity even in CF patients with normal FEV_1 and has therefore emerged as an endpoint measure in clinical trials of pediatric patients with normal spirometry (12-17, 19), but has so far received little attention for the assessment of lung function in adult CF patients with impaired spirometry. In clinical trials in Phe508del homozygous children aged 6-11 years with well-preserved spirometry, LCI was improved by lumacaftor-ivacaftor after 24 weeks by 1.0 and 0.88 respectively (16, 17). In our study in adolescents and adults, mean FEV_1 % predicted was ~65% with most patients showing a moderate to severe impairment in spirometry (Table 1 and Figure 1). In our patients with advanced lung disease and impaired spirometry, LCI improved under lumacaftor-ivacaftor therapy (Figure 1). The absolute improvement in LCI in our cohort was ~2-fold greater and the relative change in LCI was similar compared to previous studies of lumacaftor-ivacaftor in populations of children and adolescents with CF with preserved spirometry (16-18). This

ANNALSATS Articles in Press. Published February 18, 2021 as 10.1513/AnnalsATS.202008-1054OC

improvement in LCI after initiation of lumacaftor-ivacaftor therapy was detected despite the lack of significant improvement in FEV_1 % predicted with the number of patients included in this real-world post-approval study (Figure 1). Similar to previous studies in children and adolescents with CF with preserved spirometry (16-18), our findings therefore suggest that LCI may also be more sensitive than FEV_1 % predicted to detect response to therapy in adult CF patients with moderate to severe limitations in spirometry, and may therefore be considered as a sensitive outcome measure in future clinical trials in this patient group.

Besides MBW as a global measure of lung function, cross-sectional lung imaging by CT or MRI has emerged as an additional approach that provides information on the nature and regional distribution of abnormalities in CF lung disease and may also serve as outcome measure to assess response to therapy in patients with CF (14, 21-23, 51). Previous studies using chest CT in CF patients with a Gly551Asp mutation showed that initiation of highly effective CFTR modulator therapy with ivacaftor reduced airway mucus and peribronchial thickening, but had no effect on bronchiectasis in this patient group (21, 22). However, effects of lumacaftor-ivacaftor in Phe508del homozygous CF patients on changes in lung morphology have not been determined. We have previously demonstrated that lung imaging with proton MRI is sensitive to detect response to antibiotic therapy for pulmonary exacerbation in patients with CF across the pediatric age range (14, 23) and therefore used this radiation-free imaging technique to assess effects of lumacaftor-ivacaftor therapy in our study. Similar to previous studies in CF patients with a Gly551Asp mutation treated with ivacaftor, overall morphological abnormalities reflected in the MRI morphology score were reduced after initiation of lumacaftor-ivacaftor in our cohort of Phe508del homozygous patients (Figure 2). However, this

ANNALSATS Articles in Press. Published February 18, 2021 as 10.1513/AnnalsATS.202008-1054OC

improvement under lumacaftor-ivacaftor was mainly determined by a reduction in pleural reactions associated with infection and inflammation of lung parenchyma, whereas no improvements were observed for mucus plugging or airway wall thickening/bronchiectasis MRI subscores (Table E3 in the online supplement). In addition to this improvement in the MRI morphology score, MRI perfusion studies detected improved lung perfusion after initiation of lumacaftor-ivacaftor therapy (Figure 2). Because the perfusion deficits observed in CF lung disease reflect hypoxic pulmonary vasoconstriction due to airway mucus plugging (14, 23), these results suggest that lumacaftor-ivacaftor may reduce mucus plugging in the small airways that is not visible and thus escaped detection by morphological imaging alone (23). However, when viewed in combination, these results suggest that lumacaftor-ivacaftor in Phe508del homozygous patients is substantially less effective in reducing mucus plugging than ivacaftor therapy in patients with a Gly551Asp mutation (21, 22). This observation is in line with the differences in efficacy of improvement in CFTR activity in patients, which was shown to be ~50% of normal CFTR function in patients with a Gly551Asp mutation compared to 10-20% in patients homozygous for the Phe508del mutation (7, 52).

Our data suggest that both LCI and MRI may be more sensitive than FEV_1% predicted to detect response to novel therapies for CF. With the emergence of highly effective CFTR modulator therapy for most patients with CF, sensitive outcome measures are needed to detect additional benefits of new therapies in early phase clinical trials with small sample sizes, including testing of novel CFTR-directed therapeutics, mucolytic, anti-inflammatory and anti-infective treatment strategies that are still needed to further improve quality of life and survival of people with CF and eventually achieve full correction of the CF basic defect (1, 53-56). In this

ANNALSATS Articles in Press. Published February 18, 2021 as 10.1513/AnnalsATS.202008-1054OC

context, sample size estimations based on the results of our study suggest that an early phase clinical trial of a new CFTR modulator in adolescents and adults with CF that adds 10 to 20 % of CFTR function would need to include 151 patients to determine therapeutic benefits if FEV_1 % predicted was used as primary outcome, whereas only 15 to 28 patients would be required if MRI global score or LCI were used as primary endpoint, respectively (Table E4 in the online supplement). These results support the development of LCI and MRI as novel sensitive endpoints in early phase clinical trials and potentially for monitoring of response to therapy in adult CF patients with chronic lung disease in the clinical setting.

Emerging evidence suggests that progressive dysbiosis of the airway microbiota is a key risk factor of lung disease severity in CF (29, 31). While infants and preschool children with CF show a diverse lung microbiome characterized by bacterial species from the oropharyngeal flora such as *Neisseria, Veillonella* and *Prevotella* (27, 41), adolescent and adult patients develop an increasingly less diverse microbiome that is dominated by pro-inflammatory pathogens such as *Pseudomonas aeruginosa* and associated with more severe airway inflammation and structural lung damage (30, 57). A decrease in the relative abundance of a pathogen could therefore result in a deceleration of disease progression. So far, studies of the effects of CFTR modulator therapy on the airway microbiome have been limited to CF patients with a Gly551Asp mutation that started treatment with ivacaftor (21, 32, 33). In some of these studies, ivacaftor therapy was associated with a reduction in the total bacterial load, as well as relative abundance of *Pseudomonas aeruginosa* and an increase of the relative abundance of *Streptococcus, Prevotella* and*, Veillonella* resulting in an increased Shannon diversity of the airway microbiome (21, 32, 33). This increase in microbiome diversity was associated with a

ANNALSATS Articles in Press. Published February 18, 2021 as 10.1513/AnnalsATS.202008-1054OC

reduction in the inflammation markers IL1-β, IL-8 and neutrophil elastase under ivacaftor therapy (21). Similar to these prior studies of ivacaftor effects in patients with a Gly551Asp mutation, we found a reduction in total bacterial load and an increase in Shannon diversity in our cohort of Phe508del homozygous patients after initiation of lumacaftor-ivacaftor (Figure 3). We did not observe changes in the relative abundance of single CF pathogens such as *Pseudomonas aeruginosa*, which may be attributed to the lower rate of patients with chronic *Pseudomonas aeruginosa* infection in our cohort (27%) compared to the previous study in patients with a Gly551Asp mutation (67%) (21). In our study, we observed a reduction in bacterial load and increase in Shannon diversity under lumacaftor-ivacaftor as well as a reduction in IL-1β, a key pro-inflammatory cytokine released from the inflammasome in response to bacterial stimuli. The mean levels of the other pro-inflammatory cytokines measured, as well as free NE activity as a marker associated with bronchiectasis and lung disease progression in CF, were not changed under lumacaftor-ivacaftor in the small patient cohort that was available for our study (Figure 4) (46-50). This somewhat weaker anti-inflammatory effect observed in our study may be related to the less effective rescue of CFTR function by lumacaftor-ivacaftor in patients homozygous for the Phe508del mutation compared to ivacaftor in patients with a Gly551Asp mutation (7, 52). Interestingly, in addition to neutrophil recruitment, IL-1β has recently been shown to be a potent stimulus for mucus hypersecretion by the airway epithelium (58). Therefore, the reduction of IL-1β under treatment with lumacaftor-ivacaftor may not only attenuate neutrophilic inflammation, but also mucus hypersecretion contributing to mucus plugging of the airways. Despite these beneficial effects, our data also demonstrate that improvement of CFTR function by lumacaftor-

ANNALSATS Articles in Press. Published February 18, 2021 as 10.1513/AnnalsATS.202008-1054OC

ivacaftor is insufficient to inhibit or revert chronic airway infection and inflammation completely in adolescent and adult CF patients with chronic lung disease. Whether a more complete resolution of the abnormal airway microbiome and inflammation can be achieved by emerging highly effective CFTR modulators (53, 54), or by early initiation of therapy prior to the onset of irreversible lung damage in young children with CF remains to be determined in future studies.

This study also has limitations. In this single center study investigating the effects of a single CFTR modulator therapy in a small number of CF patients presenting with a large range of age and lung disease severity (Table 1), we observed high inter-patient variability across the different endpoints. Although age and lung function at baseline were not identified as confounders, these patients characteristics may account for some of the observed variability in this small patient cohort that was available for these studies at our CF center. Further, due to the small sample size and limited overlap between patients with paired MRI and sputum microbiome studies, we were not able to determine correlations between the different endpoints assessed in our study. Since we only included patients with expectorated sputum in the airway microbiome and inflammation substudy, these patients tended to be older and to have more severe airflow limitation by spirometry than the overall cohort, potentially limiting generalizability. Therefore, future multicenter studies with these endpoints in a larger patient cohort with a more defined spectrum of age and disease severity, including longer time points and ideally also patients treated with more effective CFTR modulators therapies, will be important to achieve a better understanding of the relationship between the timing and

ANNALSATS Articles in Press. Published February 18, 2021 as 10.1513/AnnalsATS.202008-1054OC

efficacy of CFTR modulator therapy and improvement/progression of airway infection, inflammation, and changes in lung structure and function in patients with CF.

In summary, this study shows for the first time that initiation of lumacaftor-ivacaftor therapy associated with activation of 10 to 20% of normal CFTR activity (7) improves ventilation homogeneity, lung morphology and perfusion as determined by MBW and MRI as well as microbial diversity, and reduces bacterial load and inflammation in adolescent and adult Phe508del homozygous CF patients with chronic lung disease. These improvements were seen in relatively small group sizes of patients even in the absence of improvement in FEV_1 % predicted. With the emergence of more efficacious CFTR modulators, these outcome measures may facilitate the detection of therapeutic efficacy in future early phase clinical trials, as well as the elucidation of the relationship between the level of functional correction of mutant CFTR, the timing of therapeutic intervention and clinical response in patients with CF.

Acknowledgements

The authors thank the patients with CF for their participation in this study, S. Hämmerling and J. Schäfer-Feterowsky for patient recruitment and S. Butz for expert technical assistance.

ANNALSATS Articles in Press. Published February 18, 2021 as 10.1513/AnnalsATS.202008-1054OC

References

1. Mall MA, Mayer-Hamblett N, Rowe SM. Cystic Fibrosis: Emergence of Highly Effective Targeted Therapeutics and Potential Clinical Implications. *Am J Respir Crit Care Med* 2020; 201: 1193-1208.

2. Konstan MW, McKone EF, Moss RB, Marigowda G, Tian S, Waltz D, Huang X, Lubarsky B, Rubin J, Millar SJ, Pasta DJ, Mayer-Hamblett N, Goss CH, Morgan W, Sawicki GS. Assessment of safety and efficacy of long-term treatment with combination lumacaftor and ivacaftor therapy in patients with cystic fibrosis homozygous for the F508del-CFTR mutation (PROGRESS): a phase 3, extension study. *Lancet Respir Med* 2017; 5: 107-118.

3. Wainwright CE, Elborn JS, Ramsey BW, Marigowda G, Huang X, Cipolli M, Colombo C, Davies JC, de BK, Flume PA, Konstan MW, McColley SA, McCoy K, McKone EF, Munck A, Ratjen F, Rowe SM, Waltz D, Boyle MP. Lumacaftor-Ivacaftor in Patients with Cystic Fibrosis Homozygous for Phe508del CFTR. *N Engl J Med* 2015; 373: 220-231.

4. Taylor-Cousar JL, Jain M, Barto TL, Haddad T, Atkinson J, Tian S, Tang R, Marigowda G, Waltz D, Pilewski J, Group VXI. Lumacaftor/ivacaftor in patients with cystic fibrosis and advanced lung disease homozygous for F508del-CFTR. *J Cyst Fibros* 2018; 17: 228-235.

5. McColley SA, Konstan MW, Ramsey BW, Stuart Elborn J, Boyle MP, Wainwright CE, Waltz D, Vera-Llonch M, Marigowda G, Jiang JG, Rubin JL. Lumacaftor/Ivacaftor reduces pulmonary exacerbations in patients irrespective of initial changes in FEV1. *J Cyst Fibros* 2019; 18: 94-101.

6. Boyle MP, Bell SC, Konstan MW, McColley SA, Rowe SM, Rietschel E, Huang X, Waltz D, Patel NR, Rodman D. A CFTR corrector (lumacaftor) and a CFTR potentiator (ivacaftor) for treatment of patients with cystic fibrosis who have a phe508del CFTR mutation: a phase 2 randomised controlled trial. *Lancet Respir Med* 2014; 2: 527-538.

7. Graeber SY, Dopfer C, Naehrlich L, Gyulumyan L, Scheuermann H, Hirtz S, Wege S, Mairbaurl H, Dorda M, Hyde R, Bagheri-Hanson A, Rueckes-Nilges C, Fischer S, Mall MA, Tummler B. Effects of Lumacaftor-Ivacaftor Therapy on Cystic Fibrosis Transmembrane Conductance Regulator Function in Phe508del Homozygous Patients with Cystic Fibrosis. *Am J Respir Crit Care Med* 2018; 197: 1433-1442.

8. Hirtz S, Gonska T, Seydewitz HH, Thomas J, Greiner P, Kuehr J, Brandis M, Eichler I, Rocha H, Lopes AI, Barreto C, Ramalho A, Amaral MD, Kunzelmann K, Mall M. CFTR Cl- channel function in native human colon correlates with the genotype and phenotype in cystic fibrosis. *Gastroenterology* 2004; 127: 1085-1095.

9. Wilschanski M, Famini C, Blau H, Rivlin J, Augarten A, Avital A, Kerem B, Kerem E. A pilot study of the effect of gentamicin on nasal potential difference measurements in cystic fibrosis patients carrying stop mutations. *Am J Respir Crit Care Med* 2000; 161: 860-865.

ANNALSATS Articles in Press. Published February 18, 2021 as 10.1513/AnnalsATS.202008-1054OC

10. Amin R, Subbarao P, Jabar A, Balkovec S, Jensen R, Kerrigan S, Gustafsson P, Ratjen F. Hypertonic saline improves the LCI in paediatric patients with CF with normal lung function. *Thorax* 2010; 65: 379-383.

11. Amin R, Subbarao P, Lou W, Jabar A, Balkovec S, Jensen R, Kerrigan S, Gustafsson P, Ratjen F. The effect of dornase alfa on ventilation inhomogeneity in patients with cystic fibrosis. *Eur Respir J* 2011; 37: 806-812.

12. Davies J, Sheridan H, Bell N, Cunningham S, Davis SD, Elborn JS, Milla CE, Starner TD, Weiner DJ, Lee PS, Ratjen F. Assessment of clinical response to ivacaftor with lung clearance index in cystic fibrosis patients with a G551D-CFTR mutation and preserved spirometry: a randomised controlled trial. *Lancet Respir Med* 2013; 1: 630-638.

13. Kent L, Reix P, Innes JA, Zielen S, Le Bourgeois M, Braggion C, Lever S, Arets HG, Brownlee K, Bradley JM, Bayfield K, O'Neill K, Savi D, Bilton D, Lindblad A, Davies JC, Sermet I, De Boeck K, European Cystic Fibrosis Society Clinical Trial Network Standardisation C. Lung clearance index: evidence for use in clinical trials in cystic fibrosis. *J Cyst Fibros* 2014; 13: 123-138.

14. Stahl M, Wielputz MO, Graeber SY, Joachim C, Sommerburg O, Kauczor HU, Puderbach M, Eichinger M, Mall MA. Comparison of Lung Clearance Index and Magnetic Resonance Imaging for Assessment of Lung Disease in Children with Cystic Fibrosis. *Am J Respir Crit Care Med* 2017; 195: 349-359.

15. Stahl M, Wielputz MO, Ricklefs I, Dopfer C, Barth S, Schlegtendal A, Graeber SY, Sommerburg O, Diekmann G, Husing J, Koerner-Rettberg C, Nahrlich L, Dittrich AM, Kopp MV, Mall MA. Preventive Inhalation of Hypertonic Saline in Infants with Cystic Fibrosis (PRESIS). A Randomized, Double-Blind, Controlled Study. *Am J Respir Crit Care Med* 2019; 199: 1238-1248.

16. Milla CE, Ratjen F, Marigowda G, Liu F, Waltz D, Rosenfeld M, Group VXPBI. Lumacaftor/Ivacaftor in Patients Aged 6-11 Years with Cystic Fibrosis and Homozygous for F508del-CFTR. *Am J Respir Crit Care Med* 2017; 195: 912-920.

17. Ratjen F, Hug C, Marigowda G, Tian S, Huang X, Stanojevic S, Milla CE, Robinson PD, Waltz D, Davies JC, group VXi. Efficacy and safety of lumacaftor and ivacaftor in patients aged 6-11 years with cystic fibrosis homozygous for F508del-CFTR: a randomised, placebo-controlled phase 3 trial. *Lancet Respir Med* 2017; 5: 557-567.

18. Shaw M, Khan U, Clancy JP, Donaldson SH, Sagel SD, Rowe SM, Ratjen F. Changes in LCI in F508del/F508del patients treated with lumacaftor/ivacaftor: Results from the prospect study. *J Cyst Fibros* 2020; S1569-1993: 30160-0.

19. Ratjen F, Davis SD, Stanojevic S, Kronmal RA, Hinckley Stukovsky KD, Jorgensen N, Rosenfeld M, Group SS. Inhaled hypertonic saline in preschool children with cystic fibrosis (SHIP): a multicentre, randomised, double-blind, placebo-controlled trial. *Lancet Respir Med* 2019; 7: 802-809.

20. Altes TA, Johnson M, Fidler M, Botfield M, Tustison NJ, Leiva-Salinas C, de Lange EE, Froh D, Mugler JP, 3rd. Use of hyperpolarized helium-3 MRI to assess response to ivacaftor treatment in patients with cystic fibrosis. *J Cyst Fibros* 2017; 16: 267-274.

ANNALSATS Articles in Press. Published February 18, 2021 as 10.1513/AnnalsATS.202008-1054OC

21. Hisert KB, Heltshe SL, Pope C, Jorth P, Wu X, Edwards RM, Radey M, Accurso FJ, Wolter DJ, Cooke G, Adam RJ, Carter S, Grogan B, Launspach JL, Donnelly SC, Gallagher CG, Bruce JE, Stoltz DA, Welsh MJ, Hoffman LR, McKone EF, Singh PK. Restoring Cystic Fibrosis Transmembrane Conductance Regulator Function Reduces Airway Bacteria and Inflammation in People with Cystic Fibrosis and Chronic Lung Infections. *Am J Respir Crit Care Med* 2017; 195: 1617-1628.

22. Ronan NJ, Einarsson GG, Twomey M, Mooney D, Mullane D, NiChroinin M, O'Callaghan G, Shanahan F, Murphy DM, O'Connor OJ, Shortt CA, Tunney MM, Eustace JA, Maher MM, Elborn JS, Plant BJ. CORK Study in Cystic Fibrosis: Sustained Improvements in Ultra-Low-Dose Chest CT Scores After CFTR Modulation With Ivacaftor. *Chest* 2018; 153: 395-403.

23. Wielputz MO, Puderbach M, Kopp-Schneider A, Stahl M, Fritzsching E, Sommerburg O, Ley S, Sumkauskaite M, Biederer J, Kauczor HU, Eichinger M, Mall MA. Magnetic resonance imaging detects changes in structure and perfusion, and response to therapy in early cystic fibrosis lung disease. *Am J Respir Crit Care Med* 2014; 189: 956-965.

24. Eichinger M, Optazaite DE, Kopp-Schneider A, Hintze C, Biederer J, Niemann A, Mall MA, Wielputz MO, Kauczor HU, Puderbach M. Morphologic and functional scoring of cystic fibrosis lung disease using MRI. *Eur J Radiol* 2012; 81: 1321-1329.

25. Wielputz MO, Eichinger M, Wege S, Eberhardt R, Mall MA, Kauczor HU, Puderbach MU, Risse F, Heussel CP, Heussel G. Midterm Reproducibility of Chest Magnetic Resonance Imaging in Adults with Clinically Stable Cystic Fibrosis and Chronic Obstructive Pulmonary Disease. *Am J Respir Crit Care Med* 2019; 200: 103-107.

26. Fodor AA, Klem ER, Gilpin DF, Elborn JS, Boucher RC, Tunney MM, Wolfgang MC. The adult cystic fibrosis airway microbiota is stable over time and infection type, and highly resilient to antibiotic treatment of exacerbations. *PLoS One* 2012; 7: e45001.

27. Muhlebach MS, Zorn BT, Esther CR, Hatch JE, Murray CP, Turkovic L, Ranganathan SC, Boucher RC, Stick SM, Wolfgang MC. Initial acquisition and succession of the cystic fibrosis lung microbiome is associated with disease progression in infants and preschool children. *PLoS Pathog* 2018; 14: e1006798.

28. Zhao J, Schloss PD, Kalikin LM, Carmody LA, Foster BK, Petrosino JF, Cavalcoli JD, VanDevanter DR, Murray S, Li JZ, Young VB, LiPuma JJ. Decade-long bacterial community dynamics in cystic fibrosis airways. *Proc Natl Acad Sci U S A* 2012; 109: 5809-5814.

29. Bevivino A, Bacci G, Drevinek P, Nelson MT, Hoffman L, Mengoni A. Deciphering the Ecology of Cystic Fibrosis Bacterial Communities: Towards Systems-Level Integration. *Trends Mol Med* 2019; 25: 1110-1122.

30. Boutin S, Graeber SY, Stahl M, Dittrich AS, Mall MA, Dalpke AH. Chronic but not intermittent infection with Pseudomonas aeruginosa is associated with global changes of the lung microbiome in cystic fibrosis. *Eur Respir J* 2017; 50: 1701086.

31. Zemanick ET, Wagner BD, Robertson CE, Ahrens RC, Chmiel JF, Clancy JP, Gibson RL, Harris WT, Kurland G, Laguna TA, McColley SA, McCoy K, Retsch-Bogart G, Sobush KT, Zeitlin PL,

ANNALSATS Articles in Press. Published February 18, 2021 as 10.1513/AnnalsATS.202008-1054OC

Stevens MJ, Accurso FJ, Sagel SD, Harris JK. Airway microbiota across age and disease spectrum in cystic fibrosis. *Eur Respir J* 2017; 50: 1700832.

32. Rowe SM, Heltshe SL, Gonska T, Donaldson SH, Borowitz D, Gelfond D, Sagel SD, Khan U, Mayer-Hamblett N, Van Dalfsen JM, Joseloff E, Ramsey BW. Clinical Mechanism of the Cystic Fibrosis Transmembrane Conductance Regulator Potentiator Ivacaftor in G551D-mediated Cystic Fibrosis. *Am J Respir Crit Care Med* 2014; 190: 175-184.
33. Harris JK, Wagner BD, Zemanick ET, Robertson CE, Stevens MJ, Heltshe SL, Rowe SM, Sagel SD. Changes in Airway Microbiome and Inflammation with Ivacaftor Treatment in Patients with Cystic Fibrosis and the G551D Mutation. *Ann Am Thorac Soc* 2020; 17: 212-220.
34. Graeber SY, Boutin S, Wielpütz MO, Joachim C, Frey DL, Wege S, Sommerburg O, Stahl M, Dalpke A, Mall MA. P478 Effects of lumacaftor-ivacaftor therapy on ling microbiome and lung disease detected by magnetic resonance imaging in F508del-homozygous patients with cystic fibrosis. *Pediatr Pulmonol* 2019; 54: S335.
35. Graeber SY, Stahl M, Joachim C, Wege S, Sommerburg O, Wielpütz MO, Mall MA. P262 Effects of lumacaftor/ivacaftor therapy on lung disease detected by magnetic resonance imaging in F508del homozygous patients with cystic fibrosis. *Journal of Cystic Fibrosis* 2019; 18: S131.
36. Boutin S, Graeber SY, Wielpütz MO, Joachim C, Wege S, Sommerburg O, Stahl M, Dalpke AH, Mall MA. P142 Effects of short-term lumacaftor/ivacaftor therapyon lung microbiome in F508del homozygous patients with cystic fibrosis. *Journal of Cystic Fibrosis* 2019; 18: S97-S98.
37. Robinson PD, Latzin P, Verbanck S, Hall GL, Horsley A, Gappa M, Thamrin C, Arets HG, Aurora P, Fuchs SI, King GG, Lum S, Macleod K, Paiva M, Pillow JJ, Ranganathan S, Ratjen F, Singer F, Sonnappa S, Stocks J, Subbarao P, Thompson BR, Gustafsson PM. Consensus statement for inert gas washout measurement using multiple- and single- breath tests. *Eur Respir J* 2013; 41: 507-522.
38. Singer F, Kieninger E, Abbas C, Yammine S, Fuchs O, Proietti E, Regamey N, Casaulta C, Frey U, Latzin P. Practicability of nitrogen multiple-breath washout measurements in a pediatric cystic fibrosis outpatient setting. *Pediatr Pulmonol* 2013; 48: 739-746.
39. Jensen R, Stanojevic S, Klingel M, Pizarro ME, Hall GL, Ramsey K, Foong R, Saunders C, Robinson PD, Webster H, Hardaker K, Kane M, Ratjen F. A Systematic Approach to Multiple Breath Nitrogen Washout Test Quality. *PLoS One* 2016; 11: e0157523.
40. Wielputz MO, von Stackelberg O, Stahl M, Jobst BJ, Eichinger M, Puderbach MU, Nahrlich L, Barth S, Schneider C, Kopp MV, Ricklefs I, Buchholz M, Tummler B, Dopfer C, Vogel-Claussen J, Kauczor HU, Mall MA. Multicentre standardisation of chest MRI as radiation-free outcome measure of lung disease in young children with cystic fibrosis. *J Cyst Fibros* 2018; 17: 518-527.
41. Boutin S, Graeber SY, Weitnauer M, Panitz J, Stahl M, Clausznitzer D, Kaderali L, Einarsson G, Tunney MM, Elborn JS, Mall MA, Dalpke AH. Comparison of microbiomes from different

ANNALSATS Articles in Press. Published February 18, 2021 as 10.1513/AnnalsATS.202008-1054OC

niches of upper and lower airways in children and adolescents with cystic fibrosis. *PLoS One* 2015; 10: e0116029.

42. Team RC. R: A language and environment for statistical computing [Internet]. *Vienna, Austria* 2014.
43. Lahti LS, J. Microbiome R package. 2012.
44. McMurdie PJ, Holmes S. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS One* 2013; 8: e61217.
45. Burgel PR, Munck A, Durieu I, Chiron R, Mely L, Prevotat A, Murris-Espin M, Porzio M, Abely M, Reix P, Marguet C, Macey J, Sermet-Gaudelus I, Corvol H, Bui S, Lemonnier L, Dehillotte C, Da Silva J, Paillasseur JL, Hubert D, French Cystic Fibrosis Reference Network Study G. Real-Life Safety and Effectiveness of Lumacaftor-Ivacaftor in Patients with Cystic Fibrosis. *Am J Respir Crit Care Med* 2020; 201: 188-197.
46. Sagel SD, Chmiel JF, Konstan MW. Sputum biomarkers of inflammation in cystic fibrosis lung disease. *Proc Am Thorac Soc* 2007; 4: 406-417.
47. Sly PD, Gangell CL, Chen L, Ware RS, Ranganathan S, Mott LS, Murray CP, Stick SM, Investigators AC. Risk factors for bronchiectasis in children with cystic fibrosis. *N Engl J Med* 2013; 368: 1963-1970.
48. Mayer-Hamblett N, Aitken ML, Accurso FJ, Kronmal RA, Konstan MW, Burns JL, Sagel SD, Ramsey BW. Association between pulmonary function and sputum biomarkers in cystic fibrosis. *Am J Respir Crit Care Med* 2007; 175: 822-828.
49. Dittrich AS, Kuhbandner I, Gehrig S, Rickert-Zacharias V, Twigg M, Wege S, Taggart CC, Herth F, Schultz C, Mall MA. Elastase activity on sputum neutrophils correlates with severity of lung disease in cystic fibrosis. *Eur Respir J* 2018; 51: 1701910.
50. Hagner M, Frey DL, Guerra M, Dittrich AS, Halls VS, Wege S, Herth FJF, Schultz C, Mall MA. New method for rapid and dynamic quantification of elastase activity on sputum neutrophils from patients with cystic fibrosis using flow cytometry. *Eur Respir J* 2020; 55: 1902355.
51. Grasemann H, Ciet P, Amin R, McDonald N, Klingel M, Tiddens H, Ratjen F, Grosse-Wortmann L. Changes in magnetic resonance imaging scores and ventilation inhomogeneity in children with cystic fibrosis pulmonary exacerbations. *Eur Respir J* 2017; 50: 1700244.
52. Graeber SY, Hug MJ, Sommerburg O, Hirtz S, Hentschel J, Heinzmann A, Dopfer C, Schulz A, Mainz JG, Tummler B, Mall MA. Intestinal Current Measurements Detect Activation of Mutant CFTR in Patients with Cystic Fibrosis with the G551D Mutation Treated with Ivacaftor. *Am J Respir Crit Care Med* 2015; 192: 1252-1255.
53. Middleton PG, Mall MA, Drevinek P, Lands LC, McKone EF, Polineni D, Ramsey BW, Taylor-Cousar JL, Tullis E, Vermeulen F, Marigowda G, McKee CM, Moskowitz SM, Nair N, Savage J, Simard C, Tian S, Waltz D, Xuan F, Rowe SM, Jain R, Group VXS. Elexacaftor-Tezacaftor-Ivacaftor for Cystic Fibrosis with a Single Phe508del Allele. *N Engl J Med* 2019; 381: 1809-1819.

ANNALSATS Articles in Press. Published February 18, 2021 as 10.1513/AnnalsATS.202008-1054OC

54. Heijerman HGM, McKone EF, Downey DG, Van Braeckel E, Rowe SM, Tullis E, Mall MA, Welter JJ, Ramsey BW, McKee CM, Marigowda G, Moskowitz SM, Waltz D, Sosnay PR, Simard C, Ahluwalia N, Xuan F, Zhang Y, Taylor-Cousar JL, McCoy KS, Group VXT. Efficacy and safety of the elexacaftor plus tezacaftor plus ivacaftor combination regimen in people with cystic fibrosis homozygous for the F508del mutation: a double-blind, randomised, phase 3 trial. *Lancet* 2019; 394: 1940-1948.

55. Bell SC, Mall MA, Gutierrez H, Macek M, Madge S, Davies JC, Burgel PR, Tullis E, Castanos C, Castellani C, Byrnes CA, Cathcart F, Chotirmall SH, Cosgriff R, Eichler I, Fajac I, Goss CH, Drevinek P, Farrell PM, Gravelle AM, Havermans T, Mayer-Hamblett N, Kashirskaya N, Kerem E, Mathew JL, McKone EF, Naehrlich L, Nasr SZ, Oates GR, O'Neill C, Pypops U, Raraigh KS, Rowe SM, Southern KW, Sivam S, Stephenson AL, Zampoli M, Ratjen F. The future of cystic fibrosis care: a global perspective. *Lancet Respir Med* 2020; 8: 65-124.

56. Gentzsch M, Mall MA. Ion Channel Modulators in Cystic Fibrosis. *Chest* 2018; 154: 383-393.

57. Caverly LJ, Zhao J, LiPuma JJ. Cystic fibrosis lung microbiome: opportunities to reconsider management of airway infection. *Pediatr Pulmonol* 2015; 50 Suppl 40: S31-38.

58. Chen G, Sun L, Kato T, Okuda K, Martino MB, Abzhanova A, Lin JM, Gilmore RC, Batson BD, O'Neal YK, Volmer AS, Dang H, Deng Y, Randell SH, Button B, Livraghi-Butrico A, Kesimer M, Ribeiro CM, O'Neal WK, Boucher RC. IL-1beta dominates the promucin secretory cytokine profile in cystic fibrosis. *J Clin Invest* 2019; 129: 4433-4450.

ANNALSATS Articles in Press. Published February 18, 2021 as 10.1513/AnnalsATS.202008-1054OC

Figure Legends

Figure 1: Effects of lumacaftor-ivacaftor therapy on sweat chloride concentration, percent predicted forced expiratory volume in one second (FEV_1 % predicted) and lung clearance index (LCI) in Phe508del homozygous patients with cystic fibrosis (CF). (A-C) Paired measurements of sweat chloride (n = 30) (A), FEV_1 % predicted (n = 30) (B), and LCI determined by nitrogen multiple breath washout (n = 28) (C) at baseline and after initiation of lumacaftor-ivacaftor (Lum/Iva) therapy. Solid lines represent the group mean and dashed lines represent the standard deviation of the mean. $^{**}P < 0.01$ and $^{***}P < 0.001$ compared with baseline.

Figure 2: Effects of lumacaftor-ivacaftor therapy on abnormalities in lung morphology and perfusion detected by magnetic resonance imaging (MRI) in Phe508del homozygous patients with cystic fibrosis (CF). (A-D) Paired studies of lung MRI at baseline and after initiation of lumacaftor-ivacaftor (Lum/Iva) therapy (n = 14). (A) Representative MRI images at baseline (top row) and after initiation of lumacaftor-ivacaftor (bottom row) of a 15-year old male patient with CF. The MRI study at baseline showed contrast-enhancing bronchiectasis / airway wall thickening and mucus plugging with high signal intensity (black arrow), pleural reactions (black arrowheads), consolidations (white arrow) and wedge-shaped perfusion abnormalities (white arrowheads). Abnormalities in lung morphology and perfusion were reduced after initiation of Lum/Iva therapy. (B-D) Summary of MRI morphology (B), MRI perfusion (C) and MRI global score (D) at baseline and after initiation of Lum/Iva therapy in Phe508del

ANNALSATS Articles in Press. Published February 18, 2021 as 10.1513/AnnalsATS.202008-1054OC

homozygous patients with CF. Solid lines represent the group mean and dashed lines represent the standard deviation of the mean. *$P < 0.05$ compared with baseline.

Figure 3: Effects of lumacaftor-ivacaftor on the airway microbiome in Phe508del homozygous patients with cystic fibrosis. (A-E) Paired analysis of sputum microbiome at baseline and after initiation of lumacaftor-ivacaftor (Lum/Iva) therapy (n=14). (A) Relative abundance of the 24 most abundant genera (maximal abundance in at least one sample > 5%). Samples are chronologically organized and first column shows relative abundance at baseline and the second under Lum/Iva therapy for each patient. The remaining genera are compiled in the group "other". (B) Total bacterial load was determined from the number of 16S copies measured by qPCR (log 10 transformed). (C) Shannon diversity was calculated with the Shannon index. (D) Richness was estimated by the number of ribosomal sequence variants observed. (E) Dominance was determined from the relative abundance of the most abundant amplicon sequence variant. Solid lines represent the group mean and dashed lines represent the standard deviation of the mean. *$P < 0.05$ compared with baseline.

Figure 4: Effect of lumacaftor-ivacaftor therapy on airway inflammation in Phe508del homozygous patients with cystic fibrosis**.** (A-E) Paired measurements of interleukin (IL)-1β (A), IL-6 (B), IL-8 (C), tumor necrosis factor (TNF)-α (D), and free neutrophil elastase (NE) activity (E) in sputum at baseline and after initiation of lumacaftor-ivacaftor (Lum/Iva) therapy (n = 14). Solid lines represent the group median and dashed lines represent 25^{th} and 75^{th} percentile. *$P < 0.05$ compared with baseline.

ANNALSATS Articles in Press. Published February 18, 2021 as 10.1513/AnnalsATS.202008-1054OC

Table 1 - Clinical characteristics at baseline and after initiation of lumacaftor-ivacaftor in Phe508del homozygous patients with cystic fibrosis

Clinical characteristic	Baseline mean ± SD (range) or n (%)	Lumacaftor-ivacaftor therapy mean ± SD (range)	Mean change ± SD (range)	*P*
Number of patients	30			
Age (years)	20.2 ± 8.1 (12.0 - 40.7)	20.5 ± 8.1 (12.3 - 41.0)	0.3 ± 0.05 (0.2 - 0.4)	
Pediatric patients (age < 18)	16 (53%)			
Sex (female)	14 (47%)			
Genotype Phe508del/Phe508del	30 (100%)			
Pancreatic insufficiency	30 (100%)			
BMI (kg/m²)	19.3 ± 3.2 (15.1 - 26.4)	19.5 ± 3.2 (15.1 - 28.1)	0.3 ± 0.7 (-1.1 - 1.7)	<0.05
FEV_1 absolute (L)	2.17 ± 0.72 (0.85 - 3.55)	2.26 ± 0.74 (0.86 - 3.71)	0.08 ± 0.24 (-0.64 - 0.53)	0.065
FEV_1 % predicted	65.4 ± 19.3 (26.9 - 98.4)	67.1 ± 19.6 (25.2 - 99.7)	1.7 ± 7.4 (-15.7 - 17.1)	0.221
VC_{max} % predicted	82.8 ± 14.9 (44.0 - 106.8)	84.7 ± 15.8 (39.5 - 118.4)	2.0 ± 7.0 (-13.7 - 15.8)	0.135
FEV_1 / VC_{max} % predicted	82.8 ± 13.2 (51.1 - 103.3)	83.1 ± 13.2 (48.4 - 105.9)	0.3 ± 4.4 (-10.5 - 5.8)	0.731
MEF_{25} % predicted	35.0 ± 24.8 (0.6 - 86.0)	38.1 ± 23.8 (9.7 - 89.6)	3.0 ± 11.3 (-29.81 - 30.0)	0.151

Definition of abbreviations: BMI = body mass index; FEV_1 = forced expiratory flow in one second; VC_{max} = maximum vital capacity; MEF_{25} = mean expiratory flow at 25% of capacity.

ANNALSATS Articles in Press. Published February 18, 2021 as 10.1513/AnnalsATS.202008-1054OC

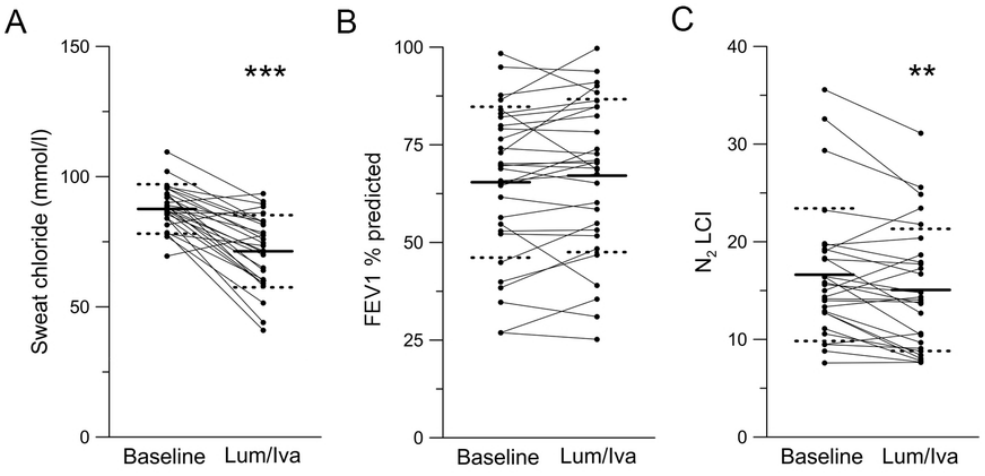

Figure 1

74x36mm (300 x 300 DPI)

ANNALSATS Articles in Press. Published February 18, 2021 as 10.1513/AnnalsATS.202008-1054OC

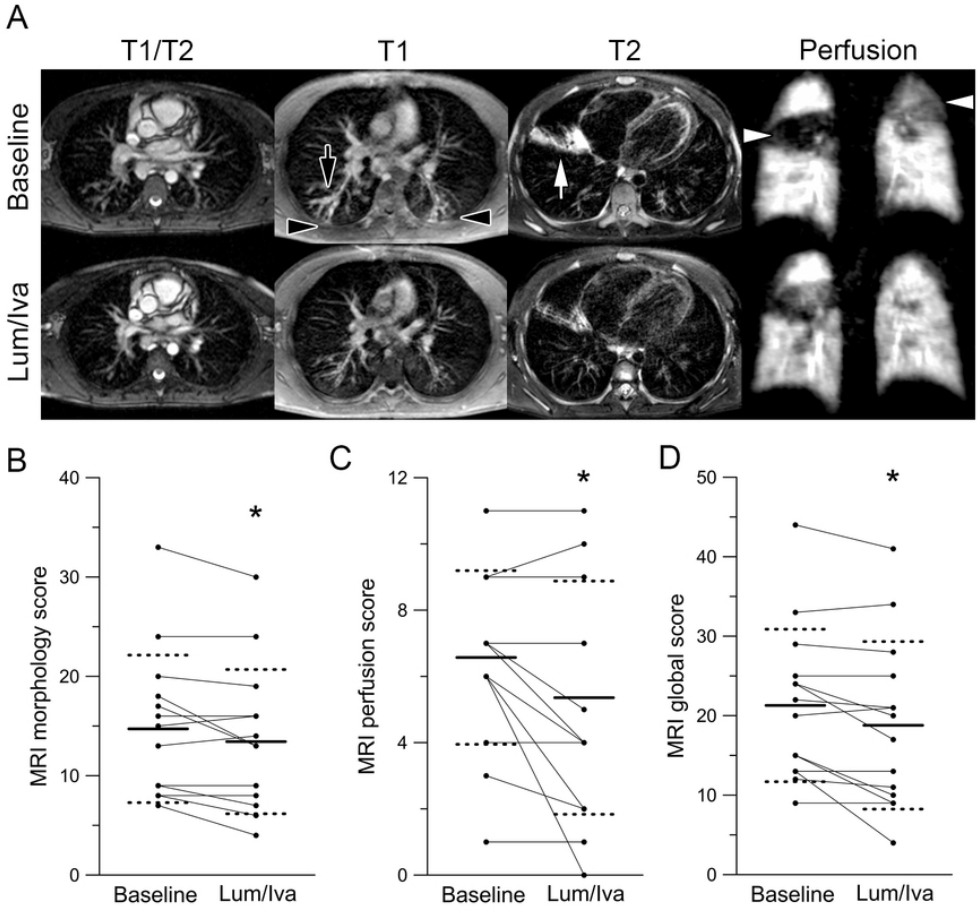

Figure 2

74x68mm (300 x 300 DPI)

ANNALSATS Articles in Press. Published February 18, 2021 as 10.1513/AnnalsATS.202008-1054OC

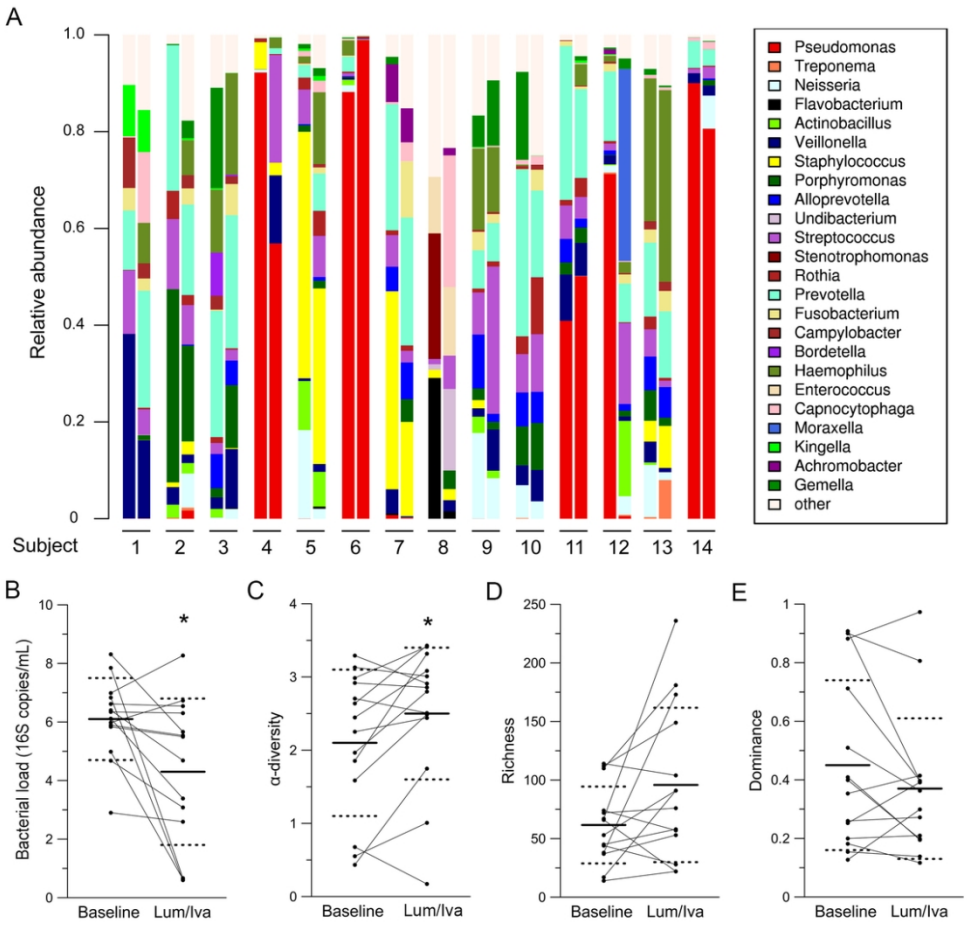

Figure 3

99x93mm (300 x 300 DPI)

ANNALSATS Articles in Press. Published February 18, 2021 as 10.1513/AnnalsATS.202008-1054OC

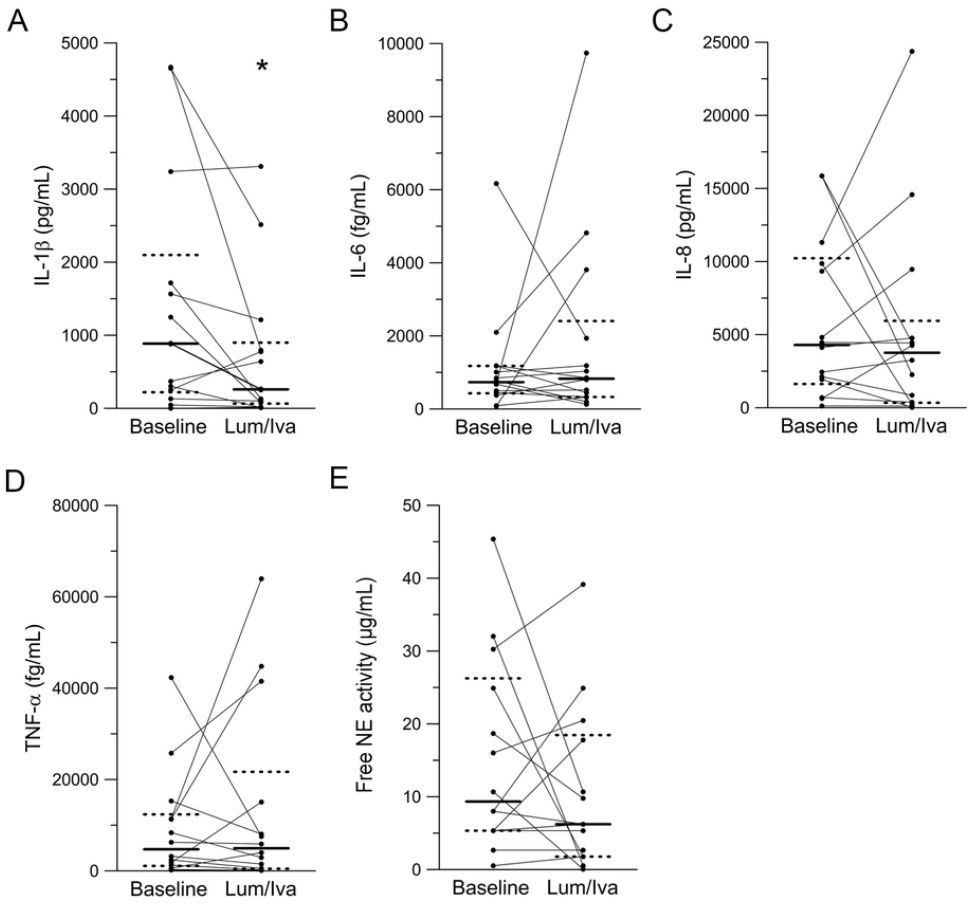

Figure 4

80x71mm (300 x 300 DPI)

ANNALSATS Articles in Press. Published February 18, 2021 as 10.1513/AnnalsATS.202008-1054OC

Effects of Lumacaftor-Ivacaftor on Lung Clearance Index, Magnetic Resonance Imaging and Airway Microbiome in Phe508del Homozygous Patients with Cystic Fibrosis

Simon Y. Graeber, Sébastien Boutin, Mark O. Wielpütz, Cornelia Joachim, Dario L. Frey, Sabine Wege, Olaf Sommerburg, Hans-Ulrich Kauczor, Mirjam Stahl, Alexander H. Dalpke, Marcus A. Mall

Online Data Supplement

ANNALSATS Articles in Press. Published February 18, 2021 as 10.1513/AnnalsATS.202008-1054OC

Supplemental Methods

Study design and participants

This prospective observational study was approved by the ethics committee of the University of Heidelberg (S-370/2011) and written informed consent was obtained from all patients, their parents or legal guardians. Patients with cystic fibrosis (CF) were eligible to participate if they were at least 12 years old, homozygous for the Phe508del mutation in the cystic fibrosis transmembrane regulator (*CFTR*) gene, had no prior exposure to lumacaftor-ivacaftor, and were willing to remain on a stable medication regimen and administration of lumacaftor-ivacaftor according to the FDA-approved patient labeling and the prescribing information for the duration of study participation. Exclusion criteria were any comorbidity that, in the opinion of the investigator, may confound the results of the study or pose an additional risk for administration of lumacaftor-ivacaftor; as well as an acute respiratory infection or pulmonary exacerbation at baseline. 30 patients were included and anthropometry, lung function, sweat chloride concentrations, multiple breath washout (MBW), lung magnetic resonance imaging (MRI) and sputum analysis were performed at baseline and 8 to 16 weeks (mean 12.2, range 8.7 to 15.9 weeks) after initiation of therapy with the approved dose of lumacaftor 400 mg in combination with ivacaftor 250 mg every 12 h. Regarding concurrent antibiotic therapy, 15 patients did not receive any antibiotics at baseline or follow up or during the observational period. Eleven patients were measured on the same antibiotic treatment at baseline and follow up. Two patients received alternating cycles between inhaled tobramycin and inhaled colistin, where one patient was measured on inhaled tobramycin at baseline and on inhaled colistin at follow up and the other one vice versa. Only two patients received an oral antibiotic therapy at follow up but not at baseline. One patient experienced a pulmonary exacerbation (E1) in between the study visits and was treated with intravenous antibiotics.

Spirometry

Spirometry was performed according to ATS/ERS standards and forced expiratory volume in one second (FEV_1), vital capacity (VC_{max}) and mid expiratory flow at 25% of VC_{max} (MEF_{25})

ANNALSATS Articles in Press. Published February 18, 2021 as 10.1513/AnnalsATS.202008-1054OC

were measured (E2). Percent predicted results were based on equations of the global lung initiative (E3).

Sweat chloride measurements

Sweating was stimulated by pilocarpine iontophoresis and samples were collected with the Macroduct® system (Model-3700, Wescor, Logan UT, USA). Sweat chloride concentration was measured in a minimum volume of 30 µl using a chloridometer (KWM 20 Chloridometer, Kreienbaum, Langenfeld, Germany) according to guidelines of the Clinical and Laboratory Standards Institute (E4).

Multiple breath washout (MBW)

MBW testing was performed with the Exhalyzer D system and spiroware 3.2.1 (Eco Medics, Duernten, Switzerland) and 100% oxygen was used to wash out resident nitrogen from the lungs as previously described (E5-7). Patients were investigated awake using a mouthpiece as interface and the same equipment as previously described (E5). The MBW equipment was leak tested and calibrated before each measurement, and the LCI was determined using spiroware 3.2.1 following evaluation criteria that were previously published (E8). All washout curves were saved, but only recordings meeting the acceptability criteria according to consensus guidelines were used to derive the LCI (E6). The LCI was determined from washout curves with no evidence of leak, sighs with release of trapped air, and with FRC and LCI measurements that showed an inter-test variation of less than 10% (for ≥ 3 tests at one timepoint) or 5% (for the minimum of two successful tests necessary for analysis). Abnormal LCI was defined as above 8.5, the upper limit of normal from age-matched healthy controls at our center.

Morpho-functional chest magnetic resonance imaging (MRI)

T1-weighted sequences before and after intravenous contrast, T2-weighted sequences, and first-pass four-dimensional (4D) perfusion imaging were acquired using a clinical 1.5T MR

ANNALSATS Articles in Press. Published February 18, 2021 as 10.1513/AnnalsATS.202008-1054OC

scanner (Magnetom Avanto, Siemens, Erlangen, Germany), and images were assessed for abnormalities in lung morphology and perfusion using a dedicated morpho-functional MRI score as previously described (E9-15). Perfusion studies were performed with intravenous administration of macrocyclic Gadolinium-based contrast medium. The MRI morphology score comprises subscores for (1) bronchial wall abnormalities (wall thickening and/or bronchiectasis), (2) mucus plugging, (3) sacculations and/or abscesses, (4) consolidations, and (5) pleural reaction including effusion. The extent of these structural abnormalities as well as abnormal perfusion are rated in each lobe as 0 (no abnormality), 1 (<50% of the lobe involved), or 2 (≥50% of the lobe involved). The MRI global score results from the sum of the MRI morphology and MRI perfusion score. In this real-world study in the clinical setting, MRI studies were limited to 14 patients, in part due to the limited availability of MRI scan time at the scheduled baseline and follow-up visits. Demographic characteristics of the MRI substudy were comparable to the overall cohort (Table 1 and Table E1 in the online supplement).

Sputum collection and microbiome analysis

Spontaneously expectorated sputum specimens were directly taken to the local microbiology lab. Samples were stored at 4°C and processed within 24h.

Sample pre-treatment. Samples were aliquoted (200 µl) and treated with PMA dye (Biotium Inc., Hayward, USA). PMA treatment modifies extracellular DNA from dead cells and avoids subsequent PCR amplification. 50 µM of PMA dye was added to the aliquot and incubated for 5 min in the dark. The samples were then exposed to light (650 Watt, 20 cm distance to the samples) on ice and shaking at 100 rpm for 5 min to cross-link PMA to DNA (E16). Viable cells were pelleted by centrifugation at 5000 x g for 10 min. Supernatant was removed and cells were recovered in 200 µl of sterile PBS and stored at -20°C until DNA extraction.

DNA extraction. DNA extractions were performed using the QIAamp Mini Kit (QIAGEN, Hilden, Germany). Protease solution (7.2 mAU) and 200 µl of Buffer AL were added to 200 µl of the sample followed by a 15 sec vortex. Samples were incubated at 56°C for 10 min and then purified according to the manufacturer's protocol. DNA was eluted by adding 100 µl of buffer

ANNALSATS Articles in Press. Published February 18, 2021 as 10.1513/AnnalsATS.202008-1054OC

AE to the column, incubation for 1 min at room temperature and centrifugation at 6000 x g for 1 min. Negative controls were performed by doing the extraction without clinical samples.

Quantitative PCR. The number of 16S copies was quantified by quantitative PCR (qPCR) using Unibac primer (forward: 5′-TGG AGC ATG TGG TTT AAT TCG A-3′; reverse: 5′-TGC GGG ACT TAA CCC AAC A-3′). PCR reactions were performed in 15 µl volumes composed of 1X Sybr-green mastermix (Life technology, Darmstadt, Germany), 50 pmol of each primer and 2 µl of DNA (or plasmid DNA standards). The thermal cycler conditions were: a first denaturation at 95°C for 20 sec, 40 amplification cycles (95°C for 3 sec, 60°C for 30 sec) and two final steps at 95°C for 15 sec and 60°C for 1 minute followed by melt curve analysis for specificity control. All reactions were performed in duplicates in a StepOnePlus Real-time PCR system (Applied Biosystems, Foster City, USA). Quantification of the 16S number of copies was performed by comparison to the cycle threshold value of a plasmid DNA standard which had been quantified by spectrophotometry.

Library preparation for next generation sequencing (NGS). DNA was amplified using universal bacterial primers flanking the V4 region (515F and 806R from (E17)). Each primer was tagged with an individual barcode (each barcode had at least 3nt differences to the others) to assign the sequences to the samples. PCR reactions were performed in 25 µl volumes composed of Q5 High-Fidelity 1X Master Mix (New England Biolabs GmbH, Germany), 25 pmol of each primer and 2 µl of DNA. The thermal cycler (Primus 25, Peqlab Biotechnologie GmbH, Germany or FlexCycler², Analytik Jena AG, Germany) conditions were: a first denaturation at 94°C for 3 min, 30 amplification cycles (94°C for 45 sec, 50°C for 1 min and 72°C for 1 min 30 sec) and a final extension at 72°C for 10 minutes. Negative controls were performed using the negative control from the extraction step and using sterile water as template. For each run of sequencing (pool of 95 samples), an internal control was performed by amplifying a mock community sample containing genomic DNA from 20 bacterial strains in equimolar (even) ribosomal RNA operon counts (HMD-782D, BEI resources, ATCC, USA). PCR products were checked by agarose gel electrophoresis (2%) for presence of amplicons. Amplicons were then purified using Agencourt AMPure XP beads (Beckman Coulter, Krefeld, Germany) following

ANNALSATS Articles in Press. Published February 18, 2021 as 10.1513/AnnalsATS.202008-1054OC

the manufacturer's instructions. Purified products were checked for quality and concentration using a ND-1000 Nanodrop instrument (Nanodrop, Wilmington, USA) and Bioanalyzer (Agilent Technologies Inc., Böblingen, Germany). An equimolar mix of all PCR products was sent to GATC biotech which performed the ligation of the sequencing adapters to the library and paired-end sequencing on an Illumina Miseq sequencing system with 250 cycles. Both negative controls were negative on the gel and library preparation resulted in no usable reads (percentage of reads in the run < 0.005% and 0 reads remained after quality control and chimera removal).*Analysis of sequences*. Sequences were analyzed with the R package dada2 (E18). Raw sequences were filtered and trimmed with the following parameters: maximum ambiguity: 0, number of expected errors for each read: 1, Truncate reads at the first instance of a quality score less than 2. Reads were then merged as contigs and checked for chimera with the default parameters. Amplicon sequence variants (ASV) were assigned to taxonomy using the Silva database (version 132). ASV assigned to eukaryotes, archae and chloroplast were removed from the analysis.

Sequencing description. In total, 2,140,327 clean and non-chimeric reads were used. The coverage of each sample was evaluated by rarefaction curves. All samples reached plateau indicating good coverage of sequencing. A total of 990 ASVs were found. The sequencing of the mock community allowed us to calculate a mean error rate for the PCR and sequencing of 4.3e-06. Files with sequencing data are publicly available (dx.doi.org/10.6084/m9.figshare.11297453)

Free neutrophil elastase activity

Quantification of neutrophil elastase activity in cell-free supernatant fractions was performed with the FRET reporter NEmo-1 (Sirius Fine Chemicals, Bremen, Germany) as previously described (E19, 20). In brief, samples were diluted 1:500 in PBS. For the readout a fluorescence plate reader (EnSpire 2300, PerkinElmer, Waltham, MA, USA) was used and the cleavage of the reporter was measured at $\lambda_{excitation}$ = 354nm, $\lambda_{emissionDonor}$ = 400nm and $\lambda_{emissionAcceptor}$ = 490nm. The Donor-Acceptor ratio was calculated by dividing the read out at

ANNALSATS Articles in Press. Published February 18, 2021 as 10.1513/AnnalsATS.202008-1054OC

400nm by the read out at 490nm and concentrations were calculated with using a standard curve.

ANNALSATS Articles in Press. Published February 18, 2021 as 10.1513/AnnalsATS.202008-1054OC

Supplemental Tables

Table E1 - Clinical characteristics of patients participating in magnetic resonance imaging substudy at baseline and after initiation of lumacaftor-ivacaftor

Clinical characteristic	Baseline mean ± SD (range) or n (%)	Lumacaftor-ivacaftor therapy mean ± SD (range)	Mean change ± SD (range)	p-value
Number of patients	14			
Age (years)	18.8 ± 8.6 (12.0 - 40.7)	19.0 ± 8.6 (12.3 - 41.0)	0.3 ± 0.05 (0.2 - 0.3)	
Pediatric patients (age < 18)	9 (64%)			
Sex (female)	8 (50%)			
Genotype Phe508del/Phe508del	14 (100%)			
Pancreatic insufficiency	14 (100%)			
BMI (kg/m²)	18.9 ± 2.7 (15.4 - 24.2)	19.2 ± 2.6 (15.6 - 24.6)	0.3 ± 0.6 (-0.7 - 1.4)	<0.05
FEV_1 absolute (L)	2.14 ± 0.64 (0.92 - 3.21)	2.24 ± 0.64 (0.86 - 3.07)	0.09 ± 0.16 (-0.17 - 0.34)	0.050
FEV_1 % predicted	69.65 ± 19.7 (26.9 - 98.4)	71.1 ± 18.8 (25.2 - 93.8)	1.5 ± 5.1 (-10.0 - 10.0)	0.306
VC_{max} % predicted	82.9 ± 16.1 (44.0 - 100.6)	84.8 ± 16.3 (39.5 - 104.6)	1.9 ± 5.7 (-6.1 - 13.8)	0.237
FEV_1 / VC_{max} % predicted	87.4 ± 11.2 (66.3 - 103.3)	87.8 ± 9.4 (69.3 - 105.9)	0.37 ± 3.8 (-6.3 - 5.8)	0.723
MEF_{25} % predicted	42.4 ± 27.6 (9.0 - 86.0)	44.6 ± 25.4 (12.3 - 89.1)	2.2 ± 12.1 (-29.8 - 18.7)	0.509

Definition of abbreviations: BMI = body mass index; FEV_1 = forced expiratory flow in one second; VC_{max} = maximum vital capacity; MEF_{25} = mean expiratory flow at 25% of capacity.

ANNALSATS Articles in Press. Published February 18, 2021 as 10.1513/AnnalsATS.202008-1054OC

Table E2 - MRI scores at baseline and after initiation of lumacaftor-ivacaftor

MRI score	Baseline mean ± SD or n (%)	Lumacaftor-ivacaftor mean ± SD or n (%)	Mean change ± SD or n (%)	*P*
Number of subjects, n	14	14		
Morpholgy				
Prevalence, n (%)	14 (100%)	14 (100%)	0 (0%)	
Score	14.7 ± 7.4	13.4 ± 7.3	-1.3 ± 1.9	<0.05
Wall thickening/bronchiectasis				
Prevalence, n (%)	14 (100%)	14 (100%)	0 (0%)	
Subscore	7.5 ± 2.0	7.3 ± 2.3	-0.2 ± 0.8	0.336
Mucus plugging				
Prevalence, n (%)	13 (93%)	12 (86%)	-1 (7%)	
Subscore	3.6 ± 2.2	3.4 ± 2.3	-0.2 ± 0.7	0.272
Absecess/sacculation				
Prevalence, n (%)	3 (21%)	4 (29%)	1 (7%)	
Subscore	0.3 ± 0.6	0.4 ± 0.6	0.1 ± 0.3	0.336
Consolidation				
Prevalence, n (%)	7 (50%)	6 (43%)	-1 (7%)	
Subscore	0.9 ± 1.2	0.4 ± 0.5	-0.4 ± 0.9	0.111
Pleural reaction				
Prevalence, n (%)	12 (86%)	10 (71%)	-2 (14%)	
Subscore	2.4 ± 2.5	1.9 ± 2.5	-0.5 ± 0.9	<0.05
Perfusion				
Prevalence, n (%)	14 (100%)	13 (93%)	-1 (7%)	
Score	6.6 ± 2.6	5.4 ± 3.5	-1.2 ± 2.0	<0.05
Global				
Prevalence, n (%)	14 (100%)	14 (100%)	0 (0%)	
Score	21.3 ± 9.6	18.8 ± 10.5	-2.5 ± 3.2	<0.05

Definition of abbreviations: MRI = magnetic resonance imaging.

ANNALSATS Articles in Press. Published February 18, 2021 as 10.1513/AnnalsATS.202008-1054OC

Table E3 - Clinical characteristics of patients participating in airway microbiome and inflammation substudy at baseline and after initiation of lumacaftor-ivacaftor

Clinical characteristic	Baseline mean ± SD (range) or n (%)	Lumacaftor-ivacaftor therapy mean ± SD (range)	Mean change ± SD (range)	p-value
Number of patients	14			
Age (years)	20.5 ± 8.7 (12.0 - 40.7)	20.8 ± 8.8 (12.3 - 41.0)	0.3 ± 0.06 (0.2 - 0.4)	
Pediatric patients (age < 18)	3 (21%)			
Sex (female)	5 (36%)			
Genotype Phe508del/Phe508del	14 (100%)			
Pancreatic insufficiency	14 (100%)			
BMI (kg/m²)	20.2 ± 3.5 (15.1 - 26.4)	20.3 ± 3.7 (15.1 - 28.1)	0.1 ± 0.8 (-1.1 - 1.7)	0.556
FEV_1 absolute (L)	2.14 ± 0.64 (0.92 - 3.21)	2.24 ± 0.64 (0.86 - 3.07)	0.04 ± 0.25 (-0.64 - 0.38)	0.587
FEV_1 % predicted	57.0 ± 15.9 (26.9 - 82.1)	58.1 ± 16.0 (31.0 - 84.7)	1.2 ± 6.6 (-15.7 - 10.0)	0.523
VC_{max} % predicted	75.5 ± 11.2 (51.1 - 88.7)	76.5 ± 9.2 (62.6 - 88.8)	1.06 ± 7.4 (-13.7 - 14.2)	0.604
FEV_1 / VC_{max} % predicted	77.7 ± 13.8 (51.1 - 100.6)	77.6 ± 15.6 (48.4 - 102.6)	-0.2 ± 3.9 (-9.1 - 5.0)	0.882
MEF_{25} % predicted	24.5 ± 20.4 (0.6 - 83.9)	27.5 ± 20.2 (9.7 - 89.6)	3.0 ± 7.2 (-14.4 - 14.6)	0.138

Definition of abbreviations: BMI = body mass index; FEV_1 = forced expiratory flow in one second; VC_{max} = maximum vital capacity; MEF_{25} = mean expiratory flow at 25% of capacity.

ANNALSATS Articles in Press. Published February 18, 2021 as 10.1513/AnnalsATS.202008-1054OC

Table E4 - Sample size calculations

Primary outcome	Effect of lumacaftor-ivacaftor mean change ± SD	Estimated sample size
FEV_1 % predicted	1.7 ± 7.4	151
Lung clearance index	-1.6 ± 2.9	28
MRI morphology score	-1.3 ± 1.9	19
MRI perfusion score	-1.2 ± 2.0	24
MRI global score	-2.5 ± 3.2	15

Estimated sample size for CFTR modulator treatment with lumacaftor-ivacaftor leading to partial rescue of CFTR function to mean levels of 10 – 20% of normal CFTR function in healthy individuals as determined from nasal potential difference (NPD) or intestinal current measurements (ICM). Definition of abbreviations: FEV_1 = forced expiratory flow in one second; MRI = magnetic resonance imaging. Sample size calculation was performed with a paired t-test with a power of 0.8 and an α-level of 0.05.

ANNALSATS Articles in Press. Published February 18, 2021 as 10.1513/AnnalsATS.202008-1054OC

Suplemental References

E1. Fuchs HJ, Borowitz DS, Christiansen DH, Morris EM, Nash ML, Ramsey BW, Rosenstein BJ, Smith AL, Wohl ME. Effect of aerosolized recombinant human DNase on exacerbations of respiratory symptoms and on pulmonary function in patients with cystic fibrosis. The Pulmozyme Study Group. *N Engl J Med* 1994; 331: 637-642.

E2. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, Crapo R, Enright P, van der Grinten CP, Gustafsson P, Jensen R, Johnson DC, MacIntyre N, McKay R, Navajas D, Pedersen OF, Pellegrino R, Viegi G, Wanger J, Force AET. Standardisation of spirometry. *Eur Respir J* 2005; 26: 319-338.

E3. Quanjer PH, Stanojevic S, Cole TJ, Baur X, Hall GL, Culver BH, Enright PL, Hankinson JL, Ip MS, Zheng J, Stocks J, Initiative ERSGLF. Multi-ethnic reference values for spirometry for the 3-95-yr age range: the global lung function 2012 equations. *Eur Respir J* 2012; 40: 1324-1343.

E4. Wayne PA. Sweat testing: sample collection and quantitative chloride analysis; approved guideline, 3rd ed. CLSI document C34-A3. *Clinical and Laboratory Standards Institute* 2009.

E5. Stahl M, Joachim C, Wielputz MO, Mall MA. Comparison of lung clearance index determined by washout of N2 and SF6 in infants and preschool children with cystic fibrosis. *J Cyst Fibros* 2019; 18: 399-406.

E6. Robinson PD, Latzin P, Verbanck S, Hall GL, Horsley A, Gappa M, Thamrin C, Arets HG, Aurora P, Fuchs SI, King GG, Lum S, Macleod K, Paiva M, Pillow JJ, Ranganathan S, Ratjen F, Singer F, Sonnappa S, Stocks J, Subbarao P, Thompson BR, Gustafsson PM. Consensus statement for inert gas washout measurement using multiple- and single-breath tests. *Eur Respir J* 2013; 41: 507-522.

E7. Singer F, Kieninger E, Abbas C, Yammine S, Fuchs O, Proietti E, Regamey N, Casaulta C, Frey U, Latzin P. Practicability of nitrogen multiple-breath washout measurements in a pediatric cystic fibrosis outpatient setting. *Pediatr Pulmonol* 2013; 48: 739-746.

E8. Jensen R, Stanojevic S, Klingel M, Pizarro ME, Hall GL, Ramsey K, Foong R, Saunders C, Robinson PD, Webster H, Hardaker K, Kane M, Ratjen F. A Systematic Approach to Multiple Breath Nitrogen Washout Test Quality. *PLoS One* 2016; 11: e0157523.

E9. Wielputz MO, Puderbach M, Kopp-Schneider A, Stahl M, Fritzsching E, Sommerburg O, Ley S, Sumkauskaite M, Biederer J, Kauczor HU, Eichinger M, Mall MA. Magnetic resonance imaging detects changes in structure and perfusion, and response to therapy in early cystic fibrosis lung disease. *Am J Respir Crit Care Med* 2014; 189: 956-965.

ANNALSATS Articles in Press. Published February 18, 2021 as 10.1513/AnnalsATS.202008-1054OC

E10. Eichinger M, Optazaite DE, Kopp-Schneider A, Hintze C, Biederer J, Niemann A, Mall MA, Wielputz MO, Kauczor HU, Puderbach M. Morphologic and functional scoring of cystic fibrosis lung disease using MRI. *Eur J Radiol* 2012; 81: 1321-1329.

E11. Leutz-Schmidt P, Stahl M, Sommerburg O, Eichinger M, Puderbach MU, Schenk JP, Alrajab A, Triphan SMF, Kauczor HU, Mall MA, Wielputz MO. Non-contrast enhanced magnetic resonance imaging detects mosaic signal intensity in early cystic fibrosis lung disease. *Eur J Radiol* 2018; 101: 178-183.

E12. Stahl M, Wielputz MO, Graeber SY, Joachim C, Sommerburg O, Kauczor HU, Puderbach M, Eichinger M, Mall MA. Comparison of Lung Clearance Index and Magnetic Resonance Imaging for Assessment of Lung Disease in Children with Cystic Fibrosis. *Am J Respir Crit Care Med* 2017; 195: 349-359.

E13. Wielputz MO, von Stackelberg O, Stahl M, Jobst BJ, Eichinger M, Puderbach MU, Nahrlich L, Barth S, Schneider C, Kopp MV, Ricklefs I, Buchholz M, Tummler B, Dopfer C, Vogel-Claussen J, Kauczor HU, Mall MA. Multicentre standardisation of chest MRI as radiation-free outcome measure of lung disease in young children with cystic fibrosis. *J Cyst Fibros* 2018; 17: 518-527.

E14. Wielputz MO, Eichinger M, Wege S, Eberhardt R, Mall MA, Kauczor HU, Puderbach MU, Risse F, Heussel CP, Heussel G. Midterm Reproducibility of Chest Magnetic Resonance Imaging in Adults with Clinically Stable Cystic Fibrosis and Chronic Obstructive Pulmonary Disease. *Am J Respir Crit Care Med* 2019; 200: 103-107.

E15. Wielputz MO, Eichinger M, Biederer J, Wege S, Stahl M, Sommerburg O, Mall MA, Kauczor HU, Puderbach M. Imaging of Cystic Fibrosis Lung Disease and Clinical Interpretation. *Rofo* 2016; 188: 834-845.

E16. Boutin S, Graeber SY, Weitnauer M, Panitz J, Stahl M, Clausznitzer D, Kaderali L, Einarsson G, Tunney MM, Elborn JS, Mall MA, Dalpke AH. Comparison of microbiomes from different niches of upper and lower airways in children and adolescents with cystic fibrosis. *PLoS One* 2015; 10: e0116029.

E17. Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Huntley J, Fierer N, Owens SM, Betley J, Fraser L, Bauer M, Gormley N, Gilbert JA, Smith G, Knight R. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *ISME J* 2012; 6: 1621-1624.

E18. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJ, Holmes SP. DADA2: High-resolution sample inference from Illumina amplicon data. *Nat Methods* 2016; 13: 581-583.

E19. Dittrich AS, Kuhbandner I, Gehrig S, Rickert-Zacharias V, Twigg M, Wege S, Taggart CC, Herth F, Schultz C, Mall MA. Elastase activity on sputum neutrophils correlates with severity of lung disease in cystic fibrosis. *Eur Respir J* 2018; 51: 1701910.

ANNALSATS Articles in Press. Published February 18, 2021 as 10.1513/AnnalsATS.202008-1054OC

E20. Hagner M, Frey DL, Guerra M, Dittrich AS, Halls VS, Wege S, Herth FJF, Schultz C, Mall MA. New method for rapid and dynamic quantification of elastase activity on sputum neutrophils from patients with cystic fibrosis using flow cytometry. *Eur Respir J* 2020; 55: 1902355.

ANNALSATS Articles in Press. Published February 18, 2021 as 10.1513/AnnalsATS.202008-1054OC