Effects of Lumacaftor/Ivacaftor on Cystic Fibrosis Disease Progression in Children 2 through 5 Years of Age Homozygous for *F508del-CFTR*: A Phase 2 Placebo-controlled Clinical Trial

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Abstract

Rationale: Lumacaftor/ivacaftor (LUM/IVA) was shown to be safe and well tolerated in children 2 through 5 years of age with cystic fibrosis (CF) homozygous for *F508del-CFTR* in a phase 3 open-label study. Improvements in sweat chloride concentration, markers of pancreatic function, and lung clearance index$_{2.5}$ (LCI$_{2.5}$), along with increases in growth parameters, suggested the potential for early disease modification with LUM/IVA treatment.

Objective: To further assess the effects of LUM/IVA on CF disease progression in children 2 through 5 years of age using chest magnetic resonance imaging (MRI).

Methods: This phase 2 study had two parts: a 48-week, randomized, double-blind, placebo-controlled treatment period in which children 2 through 5 years of age with CF homozygous for *F508del-CFTR* received either LUM/IVA or placebo (Part 1) followed by an open-label period in which all children received LUM/IVA for an additional 48 weeks (Part 2). We report results from Part 1. The primary endpoint was absolute change from baseline in chest MRI global score at Week 48. Secondary endpoints included absolute change in LCI$_{2.5}$ through Week 48 and absolute changes in weight-for-age, stature-for-age, and body mass index-for-age z-scores at Week 48. Additional endpoints included absolute changes in sweat chloride concentration, fecal elastase-1 levels, serum immunoreactive trypsinogen, and fecal calprotectin through Week 48. The primary endpoint was analyzed using Bayesian methods, where the actual Bayesian posterior probability of LUM/IVA being superior to placebo in the MRI global chest score at Week 48 was calculated using a vague normal prior distribution; secondary and additional endpoints were analyzed using descriptive summary statistics.

Results: Fifty-one children were enrolled and received LUM/IVA (n=35) or placebo (n=16). For the change in MRI global chest score at Week 48, the Bayesian posterior probability of LUM/IVA being better than placebo (treatment difference <0; higher score indicating greater abnormality) was 76%; the mean treatment difference was −1.5 (95% credible interval, −5.5 to 2.6). Treatment with LUM/IVA also led to within-group numerical improvements in $LCI_{2.5}$, growth parameters, and biomarkers of pancreatic function as well as greater decreases in sweat chloride concentration compared with placebo from baseline through Week 48. Safety data were consistent with the established safety profile of LUM/IVA.

Conclusions: This placebo-controlled study suggests the potential for early disease modification with LUM/IVA treatment, including that assessed by chest MRI, in children as young as 2 years.

Clinical trial registered with ClinicalTrials.gov (NCT03625466).

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Cystic fibrosis (CF) is a life-shortening, multisystemic genetic disease affecting >80,000

individuals worldwide (1). CF is caused by mutations in the gene encoding the CF

transmembrane conductance regulator (CFTR) that reduce the quantity and/or function of CFTR

protein, resulting in reduced anion transport in many epithelial organs (1, 2). *F508del-CFTR* is

the most common *CFTR* mutation, and approximately 40% of people with CF are homozygous

for *F508del* (*F/F* genotype) (3).

CFTR modulators address the underlying cause of CF by restoring CFTR activity,

modifying disease progression, and preventing or delaying organ damage (4-6). CFTR correctors

(e.g., lumacaftor, tezacaftor, and elexacaftor) improve the processing and trafficking of CFTR

protein to cell surfaces (1). CFTR potentiators (e.g., ivacaftor) increase the open probability of

CFTR channels on cell surfaces (1). Lumacaftor in combination with ivacaftor (LUM/IVA) is

currently the only CFTR modulator regimen approved for children as young as 2 years with CF

and the *F/F* genotype (7). An open-label trial showed that treatment with LUM/IVA for 24

weeks in children 2 through 5 years of age was generally safe and well tolerated and showed

within-group improvements in sweat chloride concentration, biomarkers of pancreatic function,

and growth parameters (8).

Children with CF develop lung disease early in life, even in the absence of respiratory

symptoms (9, 10). Airway infection, inflammation, and structural changes have been reported

in infants as young as 3 months (9, 11, 12). However, it remains challenging to assess lung

disease in children younger than 5 years (13).

Chest magnetic resonance imaging (MRI) allows for sensitive lung investigation, without

the radiation exposure associated with other forms of imaging (14-16). Chest MRI can detect

abnormalities in lung structure and function, such as regional mucus plugging, and has been shown to correlate with measures of lung clearance index (LCI) (17). Additionally, chest MRI can detect abnormalities in lung structure and perfusion and progression of lung disease in preschool children with CF (18, 19), as well as being able to detect response to therapy for pulmonary exacerbations (PEx) in pediatric patients with CF (17, 18).

Here, we present results from the first part of a 2-part, 96-week study of LUM/IVA in children 2 through 5 years of age with CF and the *F/F* genotype. This is the first placebo-controlled study of LUM/IVA in this age population and the first to use chest MRI as an outcome measure. Some of the results have been previously reported in the form of abstracts (20).

Methods

Participants, trial design, oversight

This phase 2, two-part study of LUM/IVA enrolled children 2 through 5 years of age with CF and the *F/F* genotype. *CFTR* genotype was confirmed at screening. Additional inclusion and exclusion criteria are provided in the online supplement.

Part 1 of the study evaluated the efficacy and impact of LUM/IVA on disease progression in a 48-week, multicenter, randomized, double-blind, placebo-controlled trial (Figure 1). A placebo control group was used in Part 1 as children were recruited for the trial before the commercial availability or approval of LUM/IVA for this age group in the region. Part 2 of the study evaluated the efficacy and safety of LUM/IVA in a 48-week open-label period. This study has completed; only Part 1 results are reported here.

LUM/IVA dosing in Part 1 was based on weight. Children were randomized (2:1) to receive either LUM/IVA (LUM 100 mg/IVA 125 mg [body weight <14 kg] or LUM 150 mg/IVA 188 mg [body weight ≥14 kg]) or matched placebo every 12 hours.

This trial was designed by Vertex Pharmaceuticals Incorporated in collaboration with the authors. The study protocol was reviewed and approved by the ethics committee of the University of Heidelberg and subsequently all participating institutions. Oversight of the safety of participating children was provided by an independent data monitoring committee. This study was conducted in accordance with the Declaration of Helsinki, local applicable laws and regulations, and current Good Clinical Practice Guidelines of the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use. Written informed consent (and assent, if applicable) was obtained from parents or legal guardians before study participation. Data collection and analysis were performed by Vertex Pharmaceuticals in collaboration with the authors. All authors had full access to trial data after final database lock, and critically reviewed the manuscript and approved the submission. Additional details regarding the chest MRI scoring system, statistical analyses, changes due to the SARS-CoV-2 pandemic, and full eligibility criteria (Table E1) are provided in the online supplement.

Outcome Measures

The primary endpoint was absolute change from baseline in the MRI global score at Week 48. A semiquantitative scoring system was used to evaluate chest MRI data (14). Each participant had 6 lobes scored with the lingula being treated as a separate lobe. A global score indicates the

level of lung abnormality (higher score indicating greater abnormality) across all parameters (bronchiectasis/wall thickening, mucus plugging, abscesses/sacculations, consolidation, special findings, and perfusion size), and morphology and perfusion scores indicate the level of abnormality across all morphological parameters and the functional parameter (14). Standardized sequence parameters were used for MRI image acquisition and are detailed in Table E2 in the online supplement. Secondary endpoints included absolute change in $LCI_{2.5}$ through Week 48 and absolute changes in weight-for-age, stature-for-age, and body mass index (BMI)-for-age z-scores at Week 48. Additional endpoints included absolute changes in sweat chloride concentration, number of PEx requiring treatment with oral or intravenous antibiotics, time to first PEx, fecal elastase-1 levels, serum immunoreactive trypsinogen, and fecal calprotectin through Week 48.

Statistical analyses

The proposed sample size of 50 children (2:1 randomization: 33 children receiving LUM/IVA and 17 children receiving placebo after adjusting for a dropout rate of 10%) was based on the number of potential children expected to be available for participation. Given there was limited information on the primary endpoint of MRI global chest score in this patient population at the time of trial design and that the sample size was based on feasibility and was not powered for between-group comparisons, Bayesian analysis and descriptive summaries of the primary endpoint were conducted. Descriptive summary statistics (n, mean, median, SD, and corresponding 95% confidence intervals [95% CI]) were provided for both within-group change and between-group difference. The Bayesian posterior probability can be interpreted as the

probability that one treatment group is better than the other (the probability that the difference between groups is <0). This posterior probability is calculated by updating prior information with new data. The actual Bayesian posterior probability of LUM/IVA being better than placebo in the chest MRI global score at Week 48 was calculated using a vague normal prior distribution. Bayesian summary statistics, including the Bayesian posterior mean difference and corresponding 95% credible intervals, were determined. Secondary efficacy endpoints were analyzed based on descriptive summary statistics (n, mean, median, SD, and corresponding 95% confidence intervals [95% CI]) for within-treatment group changes. For secondary efficacy endpoints, if either the LUM/IVA group or the placebo group demonstrated a within-treatment group change with 95% CI excluding 0, and the variability of this variable was within expectation, analysis of between-treatment group differences was also performed based on descriptive summary statistics, with corresponding 95% CIs. Additional endpoints were analyzed based on descriptive summary statistics for within-treatment group changes.

Results

Study Population

Part 1 of the study was conducted at five hospitals in Germany between August 10, 2018, and October 9, 2020. Overall, 51 children were enrolled and received at least one dose of study drug, 35 in the LUM/IVA group and 16 in the placebo group (Figure 2). Mean (SD) exposure was 47.0 (8.5) weeks in the LUM/IVA group and 48.7 (2.3) weeks in the placebo group. Two children (5.7%) discontinued LUM/IVA; one due to an adverse event (AE) that began before the first

dose of study drug and one due to starting treatment with commercially available LUM/IVA.

There were no treatment discontinuations in the placebo group. Demographics and baseline

characteristics were similar between treatment groups (Table 1).

As a result of travel restrictions due to the SARS-CoV-2 pandemic, six children missed

the Week 24 visit (LUM/IVA, n=6), five children missed the Week 36 visit (LUM/IVA, n=2;

placebo, n=3), and one child missed the Week 48 visit (LUM/IVA, n=1).

Efficacy

Treatment with LUM/IVA led to a mean absolute change in the MRI global score primary

endpoint (negative value = improvement) of −1.7 (standard deviation [SD], 6.6) compared with

−0.3 (SD, 6.1) for the placebo group from baseline at Week 48 (treatment difference −1.5; 95%

credibility interval, −5.5 to 2.6) with the Bayesian posterior probability of LUM/IVA being better

than placebo of 76% (Table 2; Figure 3 A-B). The MRI global score is defined as the sum of the 7

scoring parameters for each of the six lobes (e.g., bronchiectasis/wall thickening, mucus

plugging, abscesses/sacculations, consolidations, special findings, mosaic pattern, and perfusion

abnormalities) (Table E3). Participants taking LUM/IVA had numerically greater decreases

versus placebo in consolidations (difference -0.2 [95% CI, -0.7 to 0.3]), mosaic pattern

(difference -0.3 [95% CI, -1.7, 1.1]), mucus plugging (difference -0.2 [95% CI, -1.4 to 1.0]), and

perfusion abnormalities (difference -1.2 [95% CI, -2.7 to 0.4]); there were no numerical

differences after 48 weeks of LUM/IVA treatment in abscesses/sacculations,

bronchiectasis/wall thickening, or special findings MRI subscores versus placebo (Table E4).

Results for secondary and additional efficacy endpoints are provided in Table 3. The mean

absolute change from baseline in LCI$_{2.5}$ through Week 48 was −0.37 (95% CI, −0.85 to 0.10) in

the LUM/IVA group and 0.32 (95% CI, −0.20 to 0.84) in the placebo group (Table 3; Figure 4A).

The mean absolute changes in weight-for-age, stature-for-age, and BMI-for-age *z*-score with

LUM/IVA treatment were 0.13 (95% CI, −0.01 to 0.27), 0.09 (95% CI, −0.05 to 0.22), and 0.20

(95% CI, −0.20 to 0.41), respectively, and −0.07 (95% CI, −0.24 to 0.11), 0.10 (95% CI, −0.04 to

0.24), and −0.24 (95% CI, −0.55 to 0.07) in the placebo group from baseline at Week 48 (Table

3). The mean (SD) absolute change from baseline in sweat chloride concentration through

Week 48 was −25.4 (95% CI, −32.0 to −18.8) mmol/L in the LUM/IVA group and 1.0 (95% CI,

−4.5 to 6.6) mmol/L in the placebo group (treatment difference −26.4 mmol/L; 95% CI, −36.5 to

−16.3 mmol/L) (Table 3; Figure 4B).The mean absolute change from baseline in fecal elastase-1

concentration through Week 48 was 37.1 (95% CI, 7.2 to 67.0) μg/g in the LUM/IVA group and

2.6 (95% CI, −3.0 to 8.2) μg/g in the placebo group. The mean absolute change from baseline in

serum IRT concentration through Week 48 was −85.5 (95% CI, −177.9 to 6.8) ng/mL in the

LUM/IVA group and −37.9 (95% CI, −75.5 to −0.2) ng/mL in the placebo group (Table 3). The

mean absolute change from baseline in fecal calprotectin concentration through Week 48 was

−133.90 (95% CI, −213.94 to −35.86) μg/g in the LUM/IVA group and 26.14 (95% CI, −139.85 to

192.12) μg/g in the placebo group.

The annualized rate of PEx events requiring treatment with oral, inhaled, or intravenous

antibiotics was 0.75 in the LUM/IVA group and 1.17 in the placebo group. The annualized rate

of PEx requiring treatment with intravenous antibiotics was 0.12 in the LUM/IVA group and

0.06 in the placebo group. The annualized rate of PEx requiring hospitalizations was 0.14 in the

LUM/IVA group and 0.06 in the placebo group. The median time to first PEx requiring treatment with oral, inhaled, or intravenous antibiotics was 38.4 weeks in the placebo group; median time could not be estimated in the LUM/IVA group because <50% of children experienced events (Figure 4C). Microbiology culture results at baseline and Week 48 are shown in Table E5.

Safety

All children had at least one treatment-emergent adverse event (TEAE), which in most were mild or moderate in severity and considered by study investigators to be unlikely or not related to study drug (Table 4). The most common AEs (≥15% of children) in the LUM/IVA group were nasopharyngitis, infective PEx of CF, cough, rhinitis, abdominal pain, and pyrexia. Serious adverse events (SAEs) occurred in 20.0% of children in the LUM/IVA group and 12.5% of children in the placebo group. SAEs were consistent with background events of CF in this young age group and were considered by study investigators to be not related or unlikely related to study drug. The only SAE that occurred in more than one child in the LUM/IVA group was infective PEx of CF (three children [8.6%]). There were no AEs that led to treatment discontinuation in either treatment group.

Based on prior experience with LUM/IVA (8, 21, 22), respiratory events and elevated transaminase levels were predefined as adverse events of interest. Treatment-emergent respiratory events (defined as chest discomfort, dyspnea, respiration abnormal, asthma, bronchial hyperreactivity, bronchospasm, and wheezing) were reported in two children (5.7%) in the LUM/IVA group and three children (18.8%) in the placebo group; no child had a respiratory event that was considered serious or led to treatment discontinuation (Table E6).

Treatment-emergent events of elevated transaminase levels were reported in three children

(8.6%) in the LUM/IVA group and no children in the placebo group (Table E7); no child had an

event of elevated transaminases that was considered serious or led to treatment

discontinuation. Four children (11.4%) in the LUM/IVA group had alanine aminotransferase or

aspartate aminotransferase levels more than five times the upper limit of normal to eight or

fewer times the upper limit of normal, and 1 child (2.9%) had alanine aminotransferase or

aspartate aminotransferase levels more than eight times the upper limit of normal. No children

in the placebo group had elevated transaminase levels more than three times the upper limit of

normal. There were no clinically meaningful trends in other laboratory or vital sign

measurements and no cataracts. Overall, these safety data were consistent with the

established safety profile of LUM/IVA.

Discussion

Here, we report the use of chest MRI as an outcome measure in the first randomized placebo-

controlled study to evaluate the efficacy and safety of LUM/IVA in children 2 through 5 years of

age with CF and the *F/F* genotype. Chest MRI has been previously shown to detect early lung

structure and perfusion abnormalities and response to therapy for exacerbations in people with

CF (17-19, 23), with MRI global scores shown to decrease in children with CF following

treatment for PEx (17, 18). These reports suggested that chest MRI is an appropriate method to

assess lung structure and function in young children for whom spirometry might not be

feasible.

Several recent studies have also shown that chest MRI can be used to detect responses to CFTR modulator treatment in patients with CF and established CF lung disease. A prospective, observational study in patients with CF age 12 years and older treated with LUM/IVA showed statistically significant improvements from baseline in both MRI morphology and MRI perfusion scores, along with improved ventilation homogeneity, lung morphology, and perfusion (24). Chest MRI was also used in several prospective, observational studies of adolescent and adult patients with CF who were taking the triple combination regimen ELX/TEZ/IVA in a real-world, post-approval setting (25-27). After 3 months of ELX/TEZ/IVA therapy, MRI results showed reductions in mucus plugging and bronchial wall thickening, along with improvements in spirometry parameters, nutritional status, and sweat chloride concentration. Taken together, these studies strongly suggest that chest MRI is a useful tool in assessing lung perfusion change and disease progression in adolescents and adults taking CFTR modulators.

In the current study, the primary efficacy endpoint was absolute change in chest MRI global score. MRI global score is the sum of MRI morphology (defined as the aggregation of bronchiectasis/wall thickening, mucus plugging, abscesses/sacculations, consolidations, special findings, mosaic pattern subscores) and MRI perfusion scores (defined as the value for the perfusion subscore) (24). Children 2 through 5 years of age given LUM/IVA had numerical decreases in both MRI morphology score and MRI perfusion score over the 48-week treatment period. A previous study suggested air trapping and perfusion abnormalities could be the earliest signs of detectable CF lung disease by MRI, even before morphological changes become evident, reflecting reversible disease (28). The decrease in the MRI perfusion score seen in

children 2 through 5 years of age in this study could indicate reductions in the amount of mucus in small airways with a diameter below the resolution of MRI causing hypoxic pulmonary vasoconstriction (15, 18). Overall, for the primary endpoint, there was a larger numerical decease in the mean chest MRI global score with LUM/IVA treatment than placebo (-1.7 vs -0.3). Because the sample size for this phase 2 study was expected to be small and there was limited information with which to predict the size of a treatment effect in this age group, a Bayesian analysis of the primary endpoint was prespecified in the study protocol to provide a measure of the likelihood of a treatment difference between LUM/IVA and placebo. The calculated Bayesian posterior probability of the mean treatment difference (-1.5 [95% credible interval, -5.5 to 2.6]) being <0 (indicating LUM/IVA treatment is better than placebo) was 76%. Taken together, these results suggest that chest MRI can be used safely in children 2 through 5 years of age to identify changes or improvements in lung structure and perfusion, strongly supporting the potential of using chest MRI as an endpoint in pediatric clinical trials of CFTR modulators to assess CF lung disease progression.

Preservation of lung function and lung structure are important goals in the management of CF, and lung disease begins early in life in people with this disease (29). The utility of the LCI, derived from multiple-breath washout testing, has been demonstrated in several studies (17, 29, 30). LCI has been shown to be a more sensitive measure of lung function than forced expiratory volume in 1 second (FEV_1) in children with CF (31, 32) and can be performed at most ages because it relies only on tidal breathing and is therefore less dependent on effort than FEV_1 (31). The LCI reflects the level of ventilation inhomogeneity in the lungs, with higher values indicating a need for more lung turnovers (cycles of inhalation and exhalation) to clear a tracer

gas from the lungs (32). Children in this study had a mean baseline $LCI_{2.5}$ of 8.86 and 8.97 in the

LUM/IVA and placebo groups, respectively, indicating established airway disease at baseline

(33). The within-group numerical improvement in $LCI_{2.5}$ observed with LUM/IVA treatment in

this study was consistent with the previous phase 3, open-label study of LUM/IVA in this

pediatric population,(8) while $LCI_{2.5}$ numerically worsened over time in the placebo group.

These results, along with the changes seen in MRI global score, add to the growing body of

evidence demonstrating subclinical lung disease develops early in children with CF and that

intervention with CFTR modulators in children 2 through 5 years of age may improve lung

function and offers the opportunity to slow lung function decline over time (8, 29, 34).

Maintaining or improving nutritional status is associated with better lung function and

longer survival in patients with CF (35). During this 48-week study, within-group numerical

increases in both weight-for-age *z*-score and BMI-for-age *z*-score were observed. It is

noteworthy that the BMI-for-age *z*-score in the LUM/IVA group improved at Week 48. Overall,

these findings are consistent with improvements in nutritional parameters that have previously

been reported in open-label studies with LUM/IVA in this age group (8).

Beyond respiratory and nutritional outcomes, changes in sweat chloride concentration

provide a direct indicator of systemic CFTR function (36). The improvement in sweat chloride

concentration observed in this study (reduction of 25.4 mmol/L from a mean baseline value of

104.0 mmol/L) demonstrates the robust effect of LUM/IVA on CFTR function in these children;

this improvement is consistent with the previous reports of LUM/IVA treatment in this age

group (8) and is substantially greater than the improvement seen in previous studies of

adolescents and adults with CF (37).

Across all *CFTR* genotypes, approximately 85% of infants with CF develop exocrine pancreatic insufficiency within the first year of life and require life-long pancreatic enzyme replacement therapy (38). At baseline, children in this randomized, controlled study had pancreatic insufficiency (fecal elastase-1 concentration <200 µg/g). Within-group mean fecal elastase-1 concentration numerically improved with LUM/IVA treatment. Many children in this study had elevated serum concentrations of immunoreactive trypsinogen, a nonspecific marker of pancreatic injury (35), at baseline that numerically decreased with LUM/IVA treatment. While these results are consistent with results detected in a previous open-label LUM/IVA study in this age group (8), additional studies in larger and younger patient populations, as well as with more efficacious next-generation CFTR modulator regimens (25, 39-43), will be needed to determine the impact of CFTR modulator therapy on rescue of pancreatic function.

The current study has several limitations. While the chest MRI score is a validated measure for assessing CF-related lung disease (14), it does not have a documented minimal clinically important difference. The small number of children with CF in this study limits the ability to definitively document improvements relative to placebo and to perform subgroup analyses (44). The number of CF treatment centers currently equipped to perform chest MRI in multicenter studies is small (45); additional studies with larger numbers of CF treatment centers equipped to perform chest MRI are needed. A study with a larger sample size may better determine the correlation between abnormalities in lung morphology and perfusion detected by chest MRI, ventilation inhomogeneity detected by LCI, and other measures of early CF lung disease and extrapulmonary manifestations. It should also be noted that the software for the EcoMedics Exhalyzer-D multiple-breath washout device used for $LCI_{2.5}$ assessment in this study

was recently updated to correct for cross-sensitivity in the oxygen and carbon dioxide sensors that would otherwise overestimate the nitrogen concentration (46). A reanalysis of datasets from 6 previous studies involving 1,036 multiple-breath washout tests found that although use of this correction algorithm did result in slightly lower LCI values, the reanalysis did not change the interpretation of the results or the significance of any observed treatment effects (47). Finally, it should be noted that as this study overlapped with the first year of the COVID-19 pandemic, a global protocol addendum was implemented that enabled in-home assessments. The safety results reported here were based on both in-clinic and in-home safety assessments; however, the efficacy results presented here were based solely on in-clinic assessments.

In conclusion, this study suggests that LUM/IVA may modify CF disease progression when administered early in life. These findings also add to our knowledge of the usefulness of chest MRI in evaluating treatment benefit in preschool children with CF and support the potential of chest MRI as an outcome measure in early-intervention clinical trials. Furthermore, the abnormal $LCI_{2.5}$ at baseline and the numerical improvements in $LCI_{2.5}$ observed with LUM/IVA add to the growing body of evidence demonstrating the presence of subclinical lung disease in young children with CF and the potential of LUM/IVA to improve subclinical lung disease (29). Taken together, our results support the clinical benefit of early treatment intervention with LUM/IVA in children ≥2 years of age with CF and the *F/F* genotype.

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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. 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.

3. Zolin A, Orenti A, van Rens J, Fox A, Krasnyk M, Cosgriff R, Hatziagorou E, Jung A, Mei-Zahav M, Storms V, Jung A. European Cystic Fibrosis Society Patient Registry Annual Report 2018. Karup, Denmark: European Cystic Fibrosis Society; 2020.

4. Sawicki GS, McKone EF, Pasta DJ, Millar SJ, Wagener JS, Johnson CA, Konstan MW. Sustained benefit from ivacaftor demonstrated by combining clinical trial and cystic fibrosis patient registry data. *Am J Respir Crit Care Med* 2015; 192: 836-842.

5. 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.

6. Bessonova L, Volkova N, Higgins M, Bengtsson L, Tian S, Simard C, Konstan MW, Sawicki GS, Sewall A, Nyangoma S, Elbert A, Marshall BC, Bilton D. Data from the US and UK cystic fibrosis registries support disease modification by CFTR modulation with ivacaftor. *Thorax* 2018; 73: 731-740.

7. Vertex Pharmaceuticals (Ireland) Limited. Orkambi (lumacaftor/ivacaftor) [summary of product characteristics]. Dublin, Ireland: Vertex Pharmaceuticals (Ireland) Limited; 2019.

8. McNamara JJ, McColley SA, Marigowda G, Liu F, Tian S, Owen CA, Stiles D, Li C, Waltz D, Wang LT, Sawicki GS. Safety, pharmacokinetics, and pharmacodynamics of lumacaftor and ivacaftor combination therapy in children aged 2-5 years with cystic fibrosis homozygous for F508del-CFTR: an open-label phase 3 study. *Lancet Respir Med* 2019; 7: 325-335.

9. Stick SM, Brennan S, Murray C, Douglas T, von Ungern-Sternberg BS, Garratt LW, Gangell CL, De Klerk N, Linnane B, Ranganathan S, Robinson P, Robertson C, Sly PD. Bronchiectasis in infants and preschool children diagnosed with cystic fibrosis after newborn screening. *J Pediatr* 2009; 155: 623-628.e621.

10. Grasemann H, Ratjen F. Early lung disease in cystic fibrosis. *Lancet Respir Med* 2013; 1: 148-157.

11. Sly PD, Gangell CL, Chen L, Ware RS, Ranganathan S, Mott LS, Murray CP, Stick SM. Risk factors for bronchiectasis in children with cystic fibrosis. *N Engl J Med* 2013; 368: 1963-1970.

12. Mott LS, Park J, Murray CP, Gangell CL, de Klerk NH, Robinson PJ, Robertson CF, Ranganathan SC, Sly PD, Stick SM, Arest CF. Progression of early structural lung disease in young children with cystic fibrosis assessed using CT. *Thorax* 2012; 67: 509-516.

13. Bayfield KJ, Douglas TA, Rosenow T, Davies JC, Elborn SJ, Mall M, Paproki A, Ratjen F, Sly PD, Smyth AR, Stick S, Wainwright CE, Robinson PD. Time to get serious about the detection and monitoring of early lung disease in cystic fibrosis. *Thorax* 2021; 76: 1255-1265.

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

15. Mall MA, Stahl M, Graeber SY, Sommerburg O, Kauczor HU, Wielputz MO. Early detection and sensitive monitoring of CF lung disease: Prospects of improved and safer imaging. *Pediatr Pulmonol* 2016; 51: S49-S60.

16. Goralski JL, Stewart NJ, Woods JC. Novel imaging techniques for cystic fibrosis lung disease. *Pediatr Pulmonol* 2021; 56 Suppl 1: S40-S54.

17. Stahl M, Wielpütz 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.

18. Wielpütz 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.

19. Stahl M, Steinke E, Graeber SY, Joachim C, Seitz C, Kauczor HU, Eichinger M, Hammerling S, Sommerburg O, Wielputz MO, Mall MA. Magnetic resonance imaging detects progression of lung disease and impact of newborn screening in preschool children with cystic fibrosis. *Am J Respir Crit Care Med* 2021; 204: 943-953.

20. Stahl M, Roehmel J, Eichinger M, Doellinger F, Naehrlich L, Kopp MV, Dittrich A-M, Lee C, Sommerburg O, Tian S, Xu T, Wu P, Joshi A, Duncan ME, Wielpütz MO, Mall MA. An exploratory study to assess the impact of lumacaftor/ivacaftor on disease progression in children 2 through 5 years of age with cystic fibrosis homozygous for *F508del-CFTR* (*F/F*). 2021: Presented at: the 44th European Cystic Fibrosis Society (ECFS) Digital Conference; 49-12 June 2021.

21. Wainwright CE, Elborn JS, Ramsey BW, Marigowda G, Huang X, Cipolli M, Colombo C, Davies JC, De Boeck K, Flume PA, Konstan MW, McColley SA, McCoy K, McKone EF, Munck A, Ratjen F, Rowe SM, Waltz D, Boyle MP, Group TS, Group TS. Lumacaftor-ivacaftor in patients with cystic fibrosis homozygous for Phe508del CFTR. *N Engl J Med* 2015; 373: 220-231.

22. 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.

23. Pennati F, Roach DJ, Clancy JP, Brody AS, Fleck RJ, Aliverti A, Woods JC. Assessment of pulmonary structure-function relationships in young children and adolescents with cystic fibrosis by multivolume proton-MRI and CT. *J Magn Reson Imaging* 2018; 48: 531-542.

24. Graeber SY, Boutin S, Wielputz MO, Joachim C, Frey DL, Wege S, Sommerburg O, Kauczor HU, Stahl M, Dalpke AH, Mall MA. Effects of lumacaftor-ivacaftor on lung clearance index, magnetic resonance imaging, and airway microbiome in Phe508del homozygous patients with cystic fibrosis. *Ann Am Thorac Soc* 2021; 18: 971-980.

25. Graeber SY, Renz DM, Stahl M, Pallenberg ST, Sommerburg O, Naehrlich L, Berges J, Dohna M, Ringshausen FC, Doellinger F, Vitzthum C, Rohmel J, Allomba C, Hammerling S, Barth S, Ruckes-Nilges C, Wielputz MO, Hansen G, Vogel-Claussen J, Tummler B, Mall MA, Dittrich AM. Effects of elexacaftor/tezacaftor/ivacaftor therapy on lung clearance index and magnetic resonance imaging in patients with cystic fibrosis and one or two F508del alleles. *Am J Respir Crit Care Med* 2022; 206: 311-320.

26. Macconi L, Galici V, Di Maurizio M, Rossi E, Taccetti G, Terlizzi V. Early effects of elexacaftor-tezacaftor-ivacaftor therapy on magnetic resonance imaging in patients with cystic fibrosis and advanced lung disease. *J Clin Med* 2022; 11.

27. Wucherpfennig L, Triphan SMF, Wege S, Kauczor HU, Heussel CP, Schmitt N, Wuennemann F, Mayer VL, Sommerburg O, Mall MA, Eichinger M, Wielputz MO. Magnetic resonance imaging detects improvements of pulmonary and paranasal sinus abnormalities in response to elexacaftor/tezacaftor/ivacaftor therapy in adults with cystic fibrosis. *J Cyst Fibros* 2022; 21: 1053-1060.

28. 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.

29. 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.

30. 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.

31. Gustafsson PM, Aurora P, Lindblad A. Evaluation of ventilation maldistribution as an early indicator of lung disease in children with cystic fibrosis. *Eur Respir J* 2003; 22: 972-979.

32. Gustafsson PM, De Jong PA, Tiddens HA, Lindblad A. Multiple-breath inert gas washout and spirometry versus structural lung disease in cystic fibrosis. *Thorax* 2008; 63: 129-134.

33. Yammine S, Singer F, Abbas C, Roos M, Latzin P. Multiple-breath washout measurements can be significantly shortened in children. *Thorax* 2013; 68: 586-587.

34. Hoppe JE, Chilvers M, Ratjen F, McNamara JJ, Owen CA, Tian S, Zahigian R, Cornell AG, McColley SA. Long-term safety of lumacaftor-ivacaftor in children aged 2-5 years with cystic fibrosis homozygous for the F508del-CFTR mutation: a multicentre, phase 3, open-label, extension study. *Lancet Respir Med* 2021; 9: 977-988.

35. O'Sullivan BP, Freedman SD. Cystic fibrosis. *Lancet* 2009; 373: 1891-1904.

36. Elborn JS. Cystic fibrosis. *Lancet* 2016; 388: 2519-2531.

37. 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.

38. Singh VK, Schwarzenberg SJ. Pancreatic insufficiency in cystic fibrosis. *J Cyst Fibros* 2017; 16: S70-S78.

39. 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.

40. 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.

41. Zemanick ET, Taylor-Cousar JL, Davies J, Gibson RL, Mall MA, McKone EF, McNally P, Ramsey BW, Rayment JH, Rowe SM, Tullis E, Ahluwalia N, Chu C, Ho T, Moskowitz SM, Noel S, Tian S, Waltz D, Weinstock TG, Xuan F, Wainwright CE, McColley SA. A phase 3 open-label study of elexacaftor/tezacaftor/ivacaftor in children 6 through 11 years of age with cystic fibrosis and at least one F508del allele. *Am J Respir Crit Care Med* 2021; 203: 1522-1532.

42. Graeber SY, Vitzthum C, Pallenberg ST, Naehrlich L, Stahl M, Rohrbach A, Drescher M, Minso R, Ringshausen FC, Rueckes-Nilges C, Klajda J, Berges J, Yu Y, Scheuermann H, Hirtz S, Sommerburg O, Dittrich AM, Tummler B, Mall MA. Effects of elexacaftor/tezacaftor/ivacaftor therapy on CFTR function in patients with cystic fibrosis and one or two F508del alleles. *Am J Respir Crit Care Med* 2022; 205: 540-549.

43. Mall MA, Brugha R, Gartner S, Legg J, Moeller A, Mondejar-Lopez P, Prais D, Pressler T, Ratjen F, Reix P, Robinson PD, Selvadurai H, Stehling F, Ahluwalia N, Arteaga-Solis E, Bruinsma BG, Jennings M, Moskowitz SM, Noel S, Tian S, Weinstock TG, Wu P, Wainwright CE, Davies JC. Efficacy and safety of elexacaftor/tezacaftor/ivacaftor in children 6 through 11 years of age with cystic fibrosis heterozygous for F508del and a minimal function mutation: a phase 3b, randomized, placebo-controlled study. *Am J Respir Crit Care Med* 2022; 206: 1361-1369.

44. McGarry ME, McColley SA. Cystic fibrosis patients of minority race and ethnicity less likely eligible for CFTR modulators based on CFTR genotype. *Pediatr Pulmonol* 2021; 56: 1496-1503.

45. Wielpütz MO, von Stackelberg O, Stahl M, Jobst BJ, Eichinger M, Puderbach MU, Nährlich L, Barth S, Schneider C, Kopp MV, Ricklefs I, Buchholz M, Tümmler 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.

46. Wyler F, Oestreich MA, Frauchiger BS, Ramsey KA, Latzin P. Correction of sensor crosstalk error in Exhalyzer D multiple-breath washout device significantly impacts outcomes in children with cystic fibrosis. *J Appl Physiol (1985)* 2021; 131: 1148-1156.

47. Robinson PD, Jensen R, Seeto RA, Stanojevic S, Saunders C, Short C, Davies JC, Ratjen F. Impact of cross-sensitivity error correction on representative nitrogen-based multiple breath washout data from clinical trials. *J Cyst Fibros* 2022; 21: e204-e207.

Table 1. Participant demographics and baseline characteristics

	Lumacaftor/ivacaftor n=35	Placebo n=16
Sex, n (%)		
Male	24 (68.6)	9 (56.3)
Female	11 (31.4)	7 (43.8)
Age, mean (SD), years	4.2 (1.0)	4.2 (1.0)
<3, n (%)	4 (11.4)	1 (6.3)
≥3, n (%)	31 (88.6)	15 (93.8)
White, n (%)	35 (100.0)	16 (100.0)
MRI global score, mean (SD)	17.6 (9.7)*	21.4 (9.3)[†]
MRI morphology score, mean (SD)	13.6 (7.3)*	17.0 (7.6)[†]
MRI perfusion score, mean (SD)	4.0 (2.8)*	4.3 (2.4)
$LCI_{2.5}$, mean (SD)	8.86 (2.01)	8.97 (2.42)
$LCI_{5.0}$, mean (SD)	5.90 (0.94)	5.94 (1.06)
BMI-for-age *z* score, mean (SD)	−0.25 (1.14)	0.06 (1.03)
Stature-for-age *z* score, mean (SD)	0.36 (1.06)	0.08 (1.24)
Weight-for-age *z* score, mean (SD)	0.06 (0.92)	0.02 (1.19)
Sweat chloride concentration, mean (SD), mmol/L	104.0 (16.7)*	100.6 (7.9)
Fecal elastase-1 concentration, mean (SD), µg/g	26.6 (77.1)[‡]	8.7 (4.9)
Immunoreactive trypsinogen concentration, mean (SD), ng/mL	173.0 (316.6)[§]	155.7 (184.7)[†]
Fecal calprotectin concentration, mean (SD), µg/g	258.79 (281.57)*	215.37 (255.42)

BMI, body mass index; $LCI_{2.5}$, lung clearance index 2.5; $LCI_{5.0}$, lung clearance index 5.0; MRI, magnetic resonance imaging. * n=34. [†] n=15. [‡] n=33. [§] n=32.

Table 2. Absolute change from baseline in chest MRI global score at Week 48

	Lumacaftor/ivacaftor n=35	Placebo n=16
Baseline, n	34	15
Mean scores (SD)	17.6 (9.7)	21.4 (9.3)
Week 48, n	32	15
Mean scores (SD)	16.0 (9.4)	21.1 (11.1)
Change from baseline at Week 48, mean (SD)	−1.7 (6.6)	−0.3 (6.1)
95% CI of mean	−4.1, 0.7	−3.7, 3.1
Bayesian posterior probability for between-treatment difference (lumacaftor/ivacaftor vs placebo) <0	76%	..
Mean treatment difference (lumacaftor/ivacaftor vs placebo), 95% credible interval	−1.5 (−5.5, 2.6)	..

CI, confidence interval; MRI, magnetic resonance imaging.

Table 3. Secondary and additional endpoints

	Lumacaftor/ivacaftor n=35	Placebo n=16
Secondary endpoints		
Absolute change from baseline through week 48, mean (95% CI)		
LCI$_{2.5}$*	−0.37 (−0.85 to 0.10)	0.32 (−0.20 to 0.84)
Absolute change from baseline at week 48, mean (95% CI))		
Weight-for-age z score	0.13 (−0.01 to 0.27)[†]	−0.07 (−0.24 to 0.11)
Stature-for-age z score	0.09 (−0.05 to 0.22)[†]	0.10 (−0.04 to 0.24)
BMI-for-age z score	0.20 (−0.02 to 0.41)[†]	−0.24 (−0.55 to 0.07)
Additional endpoints		
Absolute change from baseline through week 48, mean (95% CI)		
Sweat chloride concentration, mmol/L[‡]	−25.4 (−32.0 to −18.8)[§]	1.0 (−4.5 to 6.6)
Lumacaftor/ivacaftor compared with placebo, mean (95% CI)	−26.4 (−36.5 to −16.3)	
Fecal elastase-1 concentration, µg/g*	37.1 (7.2 to 67.0)[§]	2.6 (−3.0 to 8.2)

Immunoreactive trypsinogen concentration, ng/mL*	−85.5 (−177.9 to 6.8)[†]	−37.9 (−75.5 to -0.20)[**]
Fecal calprotectin concentration, µg/g*	−133.90 (−231.94 to −35.86)[††]	26.14 (−135.85 to 192.12)
$LCI_{5.0}$*	−0.20 (−0.41 to 0.02)	0.07 (−0.20 to 0.33)
Absolute change from baseline at week 48, mean (95% CI)		
Chest MRI morphology score	−1.1 (−2.7 to 0.6)[†]	−0.9 (−3.5 to 1.7)[**]
Chest MRI perfusion score	−0.7 (−1.6 to 0.3)[†]	0.5 (−0.9 to 1.9)
Weight, kg	2.4 (2.1 to 2.8)[†]	1.9 (1.6 to 2.1)
Stature, cm	6.9 (6.3 to 7.6)[†]	6.9 (6.1 to 7.6)
BMI	0.07 (−0.17 to 0.30)[†]	−0.36 (−0.68 to −0.03)
PEx requiring oral, inhaled, or intravenous antibiotics		
Total number of patient-years	34.7	16.3
Number of children with events, n (%)	15 (42.9)	10 (62.5)
Number of events	26	19
Observed event rate per year	0.75	1.17
PEx requiring intravenous antibiotics		

Number of children with events, n (%)	4 (11.4)	1 (6.3)
Number of events	4	1
Observed event rate per year in study	0.12	0.06
PEx requiring hospitalization		
Number of children with events, n (%)	5 (14.3)	1 (6.3)
Number of events	5	1
Observed event rate per year in study	0.14	0.06

BMI, body mass index; CI, confidence interval; $LCI_{2.5}$, lung clearance index 2.5; $LCI_{5.0}$, lung clearance index 5.0; MRI, magnetic resonance imaging; PEx, pulmonary exacerbation.

* Analyzed through week 48 (average of weeks 12, 24, 36, and 48). [†] n=32. [‡] Analyzed through week 48 (average of weeks 24 and 48). [§] n=33.   The lumacaftor/ivacaftor treatment arm demonstrated a within-treatment arm change from baseline in sweat chloride concentration through week 48 with a 95% CI excluding 0; per the statistical analysis plan, the placebo-adjusted mean change was thus calculated. ** n=15. [††] n=34.

Table 4. Summary of TEAEs

	Lumacaftor/ivacaftor n=35	Placebo n=16
Any TEAEs	35 (100.0)	16 (100.0)
TEAEs by preferred term (≥15% of children)		
Nasopharyngitis	22 (62.9)	8 (50.0)
Infective PEx of CF	16 (45.7)	9 (56.3)
Cough	10 (28.6)	5 (31.3)
Rhinitis	9 (25.7)	6 (37.5)
Abdominal pain	7 (20.0)	2 (12.5)
Pyrexia	6 (17.1)	3 (18.8)
Upper respiratory tract infection	1 (2.9)	3 (18.8)
Nasal congestion	0	4 (25.0)
TEAEs by maximum severity		
Mild	10 (28.6)	6 (37.5)
Moderate	24 (68.6)	10 (62.5)
Severe	1 (2.9)	0
Life threatening	0	0
TEAEs by strongest relationship to study drug		
Related	0	0
Possibly related	13 (37.1)	8 (50.0)

Unlikely related	9 (25.7)	4 (25.0)
Not related	13 (37.1)	4 (25.0)
Serious TEAEs	7 (20.0)	2 (12.5)
Serious TEAEs (≥1% of children)		
Infective PEx of CF	3 (8.6)	1 (6.3)
Pneumonia	1 (2.9)	0
Constipation	1 (2.9)	0
Hematemesis	1 (2.9)	0
Intussusception	1 (2.9)	0
Lung infiltration	0	1 (6.3)
Related serious TEAEs	0	0
TEAEs leading to treatment interruption	3 (8.6)	0
Intussusception	1 (2.9)	0
Autoimmune hepatitis	1 (2.9)	0
Aspartate aminotransferase increase	1 (2.9)	0
TEAEs leading to treatment discontinuation	0	0
TEAEs leading to death	0	0

CF, cystic fibrosis; PEx, pulmonary exacerbation; TEAE, treatment-emergent adverse event.

Figure Legends:

Figure 1. Study design. Children were randomized 2:1 to receive either lumacaftor/ivacaftor (LUM/IVA) or placebo for up to 48 weeks (study Part 1); children in Part 2 of the study received lumacaftor/ivacaftor for up to 48 weeks. * The safety follow-up visit is scheduled to occur two weeks (± four days) after the last dose. The safety follow-up visit is required for children who complete their early-termination-of-treatment visit less than ten days after the last dose of study drug and children who interrupt study drug treatment and complete their week 96 visit less than ten days after the true last dose of study drug. It is not required for children who continue onto commercially available physician-prescribed study drug within two weeks (± four days) of completing study drug treatment at the Week 96 or early-termination-of-treatment visit.

Figure 2. Disposition of 51 children who were randomized in Part 1 of this study. * One child discontinued due to an adverse event that began prior to the first dose of study treatment.

Figure 3. (A) Representative magnetic resonance imaging (MRI) results from children with cystic fibrosis given placebo or lumacaftor/ivacaftor (LUM/IVA) at baseline and after 48 weeks of treatment. Bronchiectasis/wall thickening are indicated by black arrows, mucus plugging by white arrows, consolidations by black arrowheads, perfusion abnormalities by white arrowheads, and mosaic pattern by asterisks. Child in the left two columns received placebo and did not show changes in the MRI global score. Note the unchanged consolidation with

adjacent pleural thickening in the middle lobe and the aggravated perfusion abnormalities at Week 48. Child in the right two columns received LUM/IVA and improved in the MRI global score by 15 points at Week 48. Note reduction in bronchiectasis/wall thickening as well as mucus plugging. Mosaic pattern and perfusion abnormalities also improved. (B) Absolute change from baseline at Week 48 in MRI global score. SE, standard error.

Figure 4. Change in lung clearance index ($LCI_{2.5}$) and sweat chloride concentration from baseline and time to first pulmonary exacerbation (PEx). (A) Absolute change from baseline in $LCI_{2.5}$ at each time point. (B) Absolute change from baseline in sweat chloride concentration at each time point. (C) Kaplan-Meier plot of time to first PEx requiring treatment with oral, inhaled, or intravenous antibiotics. Data are means, and error bars are SD in panels A and B. Data are the proportion of event-free children in panel C.

Figure 1. Study design. Children were randomized 2:1 to receive either lumacaftor/ivacaftor (LUM/IVA) or placebo for up to 48 weeks (study Part 1); children in Part 2 of the study received lumacaftor/ivacaftor for up to 48 weeks. * The safety follow-up visit is scheduled to occur two weeks (± four days) after the last dose. The safety follow-up visit is required for children who complete their early-termination-of-treatment visit less than ten days after the last dose of study drug and children who interrupt study drug treatment and complete their week 96 visit less than ten days after the true last dose of study drug. It is not required for children who continue onto commercially available physician-prescribed study drug within two weeks (± four days) of completing study drug treatment at the Week 96 or early-termination-of-treatment visit.

186x68mm (300 x 300 DPI)

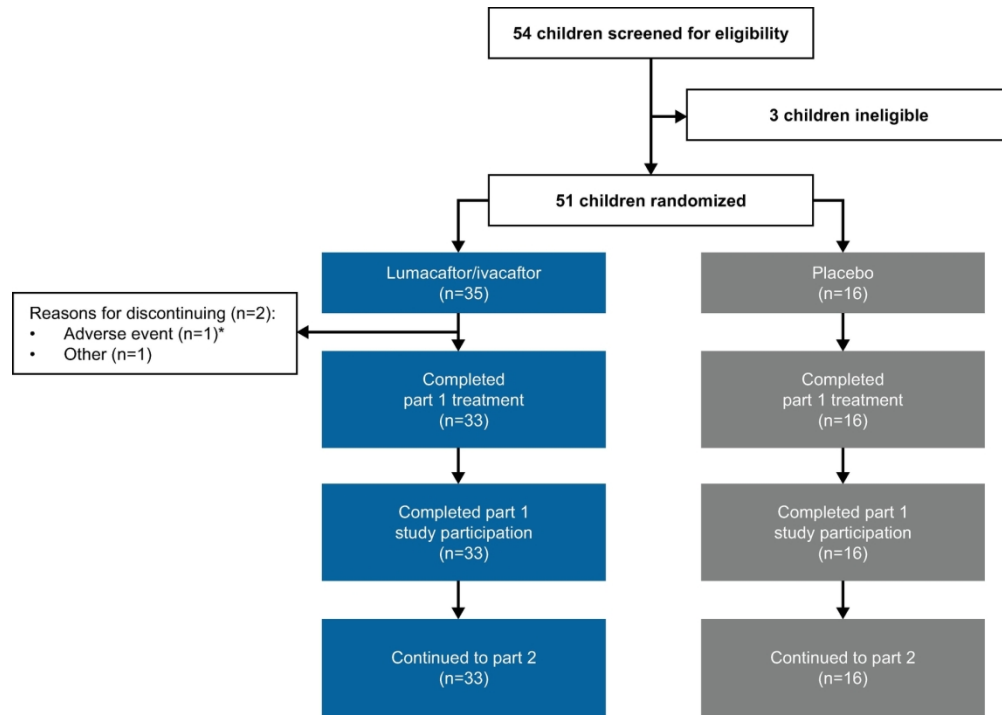Figure 2. Disposition of 51 children who were randomized in Part 1 of this study.
* One child discontinued due to an adverse event that began prior to the first dose of study treatment.

165x116mm (300 x 300 DPI)

Figure 3. (A) Representative magnetic resonance imaging (MRI) results from children with cystic fibrosis given placebo or lumacaftor/ivacaftor (LUM/IVA) at baseline and after 48 weeks of treatment. Bronchiectasis/wall thickening are indicated by black arrows, mucus plugging by white arrows, consolidations by black arrowheads, perfusion abnormalities by white arrowheads, and mosaic pattern by asterisks. Child in the left two columns received placebo and did not show changes in the MRI global score. Note the unchanged consolidation with adjacent pleural thickening in the middle lobe and the aggravated perfusion abnormalities at Week 48. Child in the right two columns received LUM/IVA and improved in the MRI global score by 15 points at Week 48. Note reduction in bronchiectasis/wall thickening as well as mucus plugging. Mosaic pattern and perfusion abnormalities also improved. (B) Absolute change from baseline at Week 48 in MRI global score. SE, standard error.

159x155mm (300 x 300 DPI)
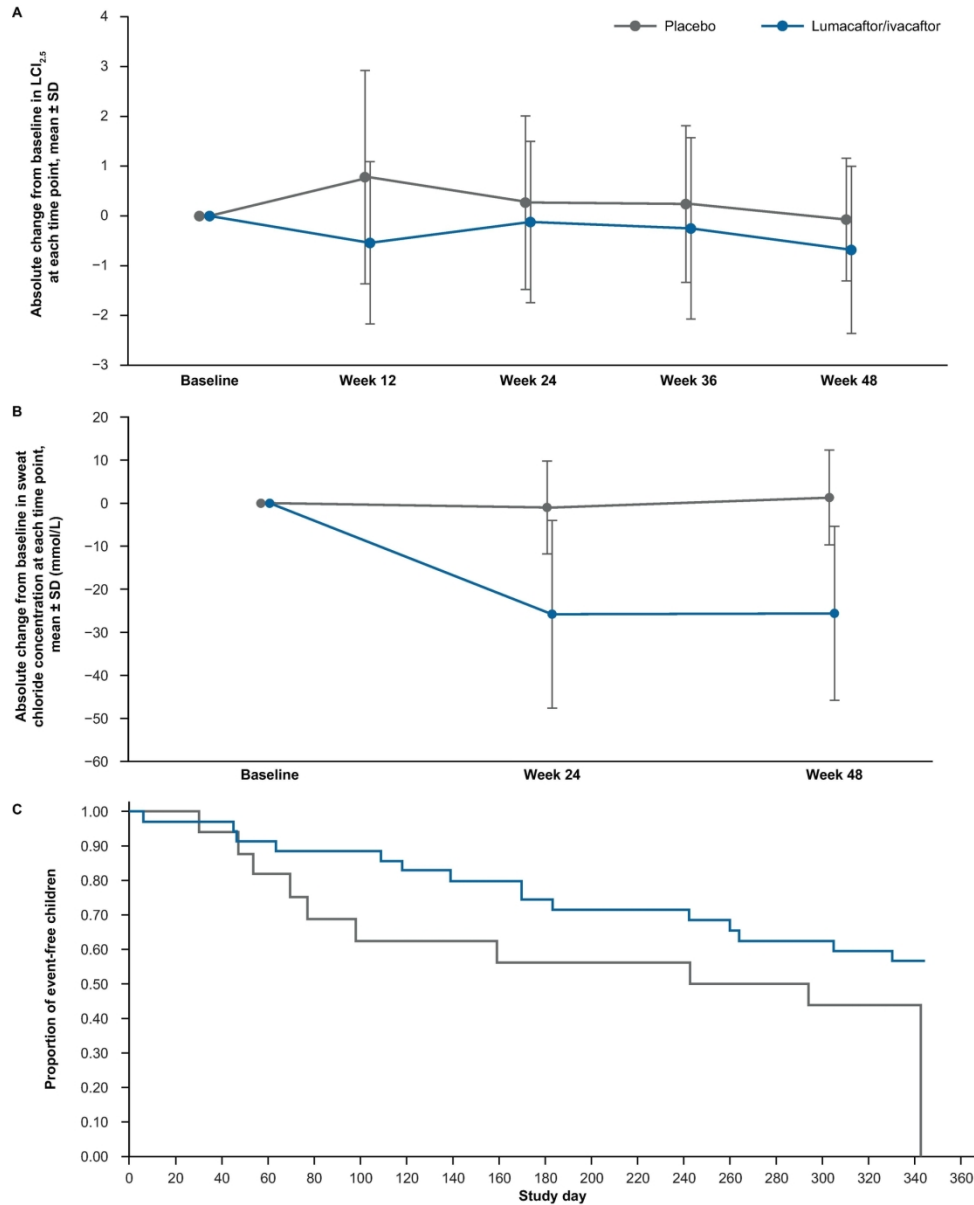
Figure 4. Change in lung clearance index (LCI2.5) and sweat chloride concentration from baseline and time to first pulmonary exacerbation (PEx). (A) Absolute change from baseline in LCI2.5 at each time point. (B) Absolute change from baseline in sweat chloride concentration at each time point. (C) Kaplan-Meier plot of time to first PEx requiring treatment with oral, inhaled, or intravenous antibiotics. Data are means, and error bars are SD in panels A and B. Data are the proportion of event-free children in panel C.

167x208mm (300 x 300 DPI)

Online Data Supplement

Effect of lumacaftor/ivacaftor on cystic fibrosis disease progression in children 2 through 5 years of age homozygous for *F508del-CFTR*: a phase 2 placebo-controlled clinical trial

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Methods

Chest magnetic resonance imaging (MRI) methods

Children were scanned in the supine position. Scans started with balanced steady-state free precession sequences (TrueFISP). Acquired in free-breathing, a negative distance factor (−50 % slice thickness) provided an overview of respiratory movements. For T1-weighted imaging fast spin echo sequence with averaging was used (T1 TSE). For T2, a half-Fourier single shot fast spin acquisition with respiratory triggering was used (T2 HASTE), combined with a T2-weighted sequence with radial phase encoding (T2 BLADE). A four-dimensional dynamic contrast-enhanced perfusion study (spoiled GRE) at high temporal resolution (1.5 s per lung volume with 35 consecutive acquisitions) with intravenous application of gadolinium-based contrast and a saline chaser by a power injector was required. Contrast materials used for this study were either Gd-DOTA/Gadoteric acid with a Gadolinium concentration of 0.5 mmol/ml (Dotarem®) or Gd-DTPA/Gadopentetat-Dimeglumine with a Gadolinium concentration of 1.0 mmol/ml (Gadovist®). Contrast dosing was performed according to the prescription information of the manufacturer. All children were sedated according to the established sedation procedure used for diagnostic procedures at each site, such as oral or rectal chloral hydrate (100 mg/kg body weight, maximum dose of 2 g) (± phenobarbital) or propofol. All children were monitored during the MRI examination by MR compatible pulse oximetry. Children were breathing spontaneously and did not require invasive ventilation during sedation.

For this study, all scanners had to be qualified. Phantom scans were used to help to ensure that the equipment is operating optimally at the acquisition parameters described in the study protocol (noted in the supplementary materials). As part of the Accreditation process, a phantom scan was performed on each MRI scanner to be used in the VX16-809-121 study. Any MRI

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phantom could be used, but it had to contain at least one water cylinder. These phantoms are usually supplied by the MRI manufacturer at the time of scanner installation for purposes of routine quality assurance testing. The phantom scan was only to be performed during the Site Accreditation process to qualify the scanner for use in the VX16-809-121 study. Though machine changes during the course of the study should have been avoided whenever possible, should a change be required, an additional phantom scan was required to qualify the new machine for the study. The images and measurements obtained from phantom scans were reviewed and assessed to ensure proper performance and adherence to imaging requirements. Any problems identified during the review were reported to the site along with recommendations to address any items of note or concern. If it was ultimately determined that a scanner was not functioning properly, it was recommended that the scanner not be used. Images obtained during the study were sent to MedQIA Imaging Services (Los Angeles, California) for scoring and QC by two radiologists who were blinded to the patients and treatment arms and who performed similar scoring in previous studies using chest MRI score(1). Images were to be submitted within 3 working days from acquisition. Protected health information was removed from all image data prior to sending to MedQIA. Overall, MRI scores were obtained from 32 out of 35 children in the LUM/IVA group and 16 out of 16 children in the placebo group at baseline and Week 48, demonstrating chest MRI in children of this age, using sedation, is possible and changes in lung function over the 48 weeks can be identified.

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Analysis of chest MRI scores

- Chest MRI scan results were assessed semiquantitatively via a standardized chest MRI scoring system (1, 2); each child had six lobes scored with the lingula treated as a separate lobe

- After scans were reviewed, chest MRI scores were captured using the following seven scoring parameters for each of the six lobes: (1) bronchiectasis/wall thickening, (2) mucus plugging, (3) abscesses/sacculations, (4) consolidations, (5) special findings, (6) mosaic pattern, and (7) perfusion abnormalities (Table E5)

- Scores assigned to each parameter were 0 = normal; 1 = <50% of lobe involved; 2 = ≥50% of lobe involved

- Aggregations of scores were defined as: MRI morphology score = sum of parameters 1–6 and MRI perfusion score = value of parameter 7

- The primary variable of MRI global score was defined as: MRI global score = sum of parameters 1–7

- The highest possible score was 84

Multiple-breath washout

The EcoMedics Exhalyzer-D multiple-breath washout (MBW) device with Spiroware version 3.2.1.21679/21680 software was used to measure nitrogen washout and thus to determine lung clearance index (LCI). The MBW testing was performed several times at each visit, and the final LCI value for each visit was calculated from the technically acceptable set of washout measurements as graded and determined by a central reader.

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Outcomes from Part 1 of the treatment cohort

Additional endpoints included absolute change from baseline in MRI morphology score, MRI perfusion score, weight, stature, body mass index, microbiology culture results, absolute change from baseline in sweat chloride concentration, number of pulmonary exacerbations (PEx) requiring treatment with oral or intravenous antibiotics, time to first PEx, cystic fibrosis-related hospitalizations, absolute change from baseline in serum concentrations of immunoreactive trypsinogen, fecal elastase-1, and fecal calprotectin, and absolute change from baseline in lung clearance index 5.0. Safety profile was assessed in terms of number of treatment-emergent adverse events, clinical laboratory values, vital signs, ophthalmologic examination findings, and physical examination findings.

Statistical analyses

The proposed sample size of 50 children (2:1 randomization: 33 in the lumacaftor/ivacaftor arm and 17 in the placebo arm) was based on the number of children expected to be available for participation.

The full analysis set consisted of randomized children with the intended *CFTR* genotype who received at least one dose of study drug in Part 1; demographics and baseline characteristics and efficacy analyses were based on the full analysis set and presented by treatment arm. The safety set included all children who received at least one dose of study drug in Part 1; safety analyses were based on the safety set and presented by treatment arm.

Within-treatment change was analyzed for secondary and additional endpoints; if either treatment arm demonstrated a within-treatment change with a 95% CI excluding 0, and the variability of this parameter was within expectation, an analysis of between-treatment difference

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was conducted based on the descriptive summary statistics with the corresponding 95% CIs.

Some data are missing because of missed visits due to the COVID-19 pandemic.

Continuous variables were summarized using descriptive statistics: the number of children (n),

mean, SD, median, minimum, and maximum, along with the corresponding 95% CIs, were

provided. Categorical variables were summarized using counts and percentages. Analysis of

number of PEx, PEx requiring treatment with intravenous antibiotics or hospitalization, and

number of planned or unplanned hospitalizations were based on descriptive summary statistics;

these included the number and percentage of children who experienced an event, total number of

events observed, total follow-up time, and observed annualized event rate. The time to first PEx

was analyzed using Kaplan-Meier estimates. Descriptive analysis was performed for safety.

Analyses were performed using SAS version 9.4.

Changes to study methods due to the SARS-CoV-2 pandemic

The sponsor implemented safety measures to provide children the opportunity to continue

participation in the study while ensuring their safety by minimizing the risk to SARS-CoV-2

exposure through travel. These operational adjustments were implemented to align with

country/local regulations and health authority guidance and based on site-level considerations

(eg, site closures due to SARS-CoV-2 or whether sites had children actively participating in the

study) to ensure the protection of children, investigators, and site personnel while maintaining

compliance with good clinical practice and minimizing impact to study integrity. Implemented

measures included use of remote consent, in-home visits by qualified personnel, and safety

assessments evaluated by telephone.

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Role of the funding source

The funder of the study had a role in study design, data collection, data analysis, data interpretation, and writing of the report. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

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Tables

Table E1. Eligibility criteria

Inclusion criteria
- Legally appointed and authorized representative (eg, parent or legal guardian) signed and dated an informed consent form, and the child signed and dated an assent form (if applicable)   - Legally appointed and authorized representative was willing and able to comply with scheduled visits, treatment plan, study restrictions, laboratory tests, and other study procedures   - Male or female and between the ages of 2 and 5 years, inclusive, on the date of informed consent (and assent, if applicable)   - Weight of ≥8 kg without shoes and wearing light clothing at the screening visit   - Confirmed diagnosis of CF, defined as   - a sweat chloride value ≥60 mmol/L by quantitative pilocarpine iontophoresis as documented in the medical record OR from the sweat chloride test result obtained at the screening visit (if an eligible historical sweat chloride result is documented in the medical record, that result alone [and not the screening visit result] may be used to determine eligibility) AND   - clinical manifestations of CF   - Homozygosity for *F508del* (*F/F*) (genotype to be confirmed at the screening visit or as documented in the medical record)   - Stable CF disease as deemed by the investigator at the screening visit

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- Willingness to remain on a stable CF medication regimen through the safety follow-up visit, if applicable

Exclusion criteria

- History of any comorbidity that, in the opinion of the investigator, might confound the results of the study or pose an additional risk in administering study drug to the child. For example:

  history of cirrhosis with portal hypertension, or prior allergic reaction to gadolinium-based contrast material, or metallic implants incompatible with MRI

- Any clinically significant laboratory abnormalities at the screening visit that would interfere with the study assessments or pose an undue risk for the child (as deemed by the investigator)

- Any of the following abnormal laboratory values at the screening visit:

  o Hemoglobin level <10 g/dL

  o Alanine aminotransferase, aspartate aminotransferase, or total bilirubin level more than two times the upper limit of normal

  o Abnormal renal function defined as glomerular filtration rate ≤45 mL/min/1.73 m^2 (calculated by the bedside Schwartz equation (3))

- Acute upper or lower respiratory tract infection, PEx as defined by the investigator, or changes in therapy (including antibiotics) for pulmonary disease within 28 days before Day one (first dose of study drug)

- Any clinically significant "non–CF-related" illness within two weeks before Day one. "Illness" is defined as an acute (serious or nonserious) condition (eg, gastroenteritis)

- History of solid organ or hematological transplant

- Ongoing or prior participation in an investigational drug study (including studies investigating lumacaftor and/or ivacaftor) within 30 days of the screening visit

  o A washout period of five terminal half-lives of the previous investigational study drug or 30 days, whichever is longer, must elapse before the screening visit

  o The duration of the elapsed time may be longer if required by local regulations. Note: Ongoing participation in a noninterventional study (including observational studies) is permitted

- Use of restricted medication or food within specified duration before the first dose of study drug

- Inability to perform the multiple-breath washout assessment during the screening period

- History of cataract/lens opacity or evidence of cataract/lens opacity determined to be clinically significant by a licensed ophthalmologist during the ophthalmologic examination at the screening visit. The screening visit ophthalmologic examination does not need to be repeated if there is documentation of an examination meeting protocol criteria that was conducted within three months before the screening visit. Children with documentation of bilateral lens removal do not need the ophthalmologic examination, and this criterion does not apply

- Previous role or a close relative in a previous role as the investigator or a subinvestigator, research assistant, pharmacist, study coordinator, or other staff directly involved with the conduct of the study

CF, cystic fibrosis; MRI, magnetic resonance imaging; PEx, pulmonary exacerbation.

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Table E2. Standardised MRI protocol

Sequence	Specials	Plane	TR (ms)	TE (ms)	ST (mm)	Distance factor (%)	Slices	FOV (mm²)	Matrix	Voxel size (mm)	Scan time (min:s)
T1/2 bSSFP		tra	339.40	1.26	4.00	-50	70	300 x 213	256 x 118	1.2 x 1.8	0:24
T1/2 bSSFP		cor	381.44	1.42	4.00	-50	67	300 x 244	256 x 168	1.2 x 1.5	0:26
T1/2 bSSFP		sag	337.00	1.26	4.00	-50	81	300 x 211	256 x 146	1.2 x 1.4	0:27
T1 FSE	4x averaging	tra	450	9.5	4.00	10	24	300 x 178	512 x 243	0.6 x 0.7	2:29
T2 FSE Half Fourier acquisition	navigated	tra	700	42	6.00	10	17	300 x 159	256 x 82	1.2 x 2.0	0:48
T2 FSE Half Fourier acquisition	navigated	cor	700	67	6.00	10	18	300 x 178	256 x 91	1.2 x 2.0	0:49
T2 FSE rotating phase encoding	navigated	tra	2330	83	4.00	15	39	400 x 400	320 x 320	1.3 x 1.3	2:14
T2 FSE rotating phase encoding	navigated	cor	2330	83	4.0	15	29	400 x 400	320 x 320	1.3 x 1.3	2:14
Perfusion	35 acquisitions, i.v. contrast	cor	2.1	0.87	5.00	3D	24	300 x 300	256 x 179	1.2 x 1.7	0:31
T1 FSE ce	4x averaging	tra	450	9.5	4.0	10	24	300 x 178	512 x 243	0.6 x 0.7	2:29
T1 FSE ce	3x averaging	cor	641	14	4.0	10	23	299 x 196	384 x 201	0.8 x 1.0	3:06

T1/2 bSSFP = balanced T1/2-weighted steady-state free-precession sequence, T1 FSE = T1-weighted fast spin echo sequence, T2 FSE = T2-weighted fast spin echo sequence, Perfusion = time-resolved 3D gradient echo sequence with parallel imaging and echo sharing for perfusion imaging acquired during contrast material injection (4D perfusion), tra = transversal plane, cor = coronal plane, sag = sagittal plane, ce = contrast enhanced, TR = repetition time, TE = echo time, ST = slice thickness, FOV = Field of view.

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Table E3. MRI global score parameters[*, (1, 2)]

Parameter	Right			Left		
	Upper lobe	Middle lobe	Lower lobe	Upper lobe	Lingula	Lower lobe
1. Bronchiectasis/wall thickening						
2. Mucus plugging						
3. Abscesses/sacculations						
4. Consolidations						
5. Special findings						
6. Mosaic pattern						
7. Perfusion abnormalities						

MRI, magnetic resonance imaging.

* Scores assigned to each parameter were 0 = normal; 1 = <50% of lobe involved; 2 = ≥50% of lobe involved.

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Table E4. Summary of MRI Subscores and Change from Baseline* at Week 48.

	Lumacaftor/ivacaftor n=35	Placebo n=16
Abscesses/Sacculations		
Baseline, mean (SD)	0.0 (0.0)	0.0 (0.0)
Week 48, mean (SD)	0.0 (0.2)	0.0 (0.0)
Absolute change at Week 48, mean (SD)	0.0 (0.2)	0.0 (0.0)
Difference vs Placebo, mean (95% CI)	0.0 (-0.1, 0.1)	
Bronchiectasis/Wall Thickening		
Baseline, mean (SD)	5.9 (1.5)	5.9 (1.5)
Week 48, mean (SD)	5.8 (1.2)	5.8 (2.2)
Absolute change at Week 48, mean (SD)	-0.1 (1.2)	-0.1 (1.1)
Difference vs Placebo, mean (95% CI)	0.0 (-0.7, 0.8)	
Consolidations		
Baseline, mean (SD)	0.6 (1.1)	0.8 (1.1)
Week 48, mean (SD)	0.4 (0.9)	0.9 (1.3)
Absolute change at Week 48, mean (SD)	-0.1 (0.5)	0.1 (1.2)
Difference vs Placebo, mean (95% CI)	-0.2 (-0.7, 0.3)	
Mosaic Pattern		
Baseline, mean (SD)	2.9 (2.9)	4.1 (2.7)
Week 48, mean (SD)	2.8 (3.0)	4.2 (2.6)
Absolute change at Week 48, mean (SD)	-0.2 (2.0)	0.1 (2.6)

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Difference vs Placebo, mean (95% CI)	-0.3 (-1.7, 1.1)	
Mucous Plugging		
Baseline, mean (SD)	3.4 (2.1)	4.4 (2.5)
Week 48, mean (SD)	3.0 (2.3)	4.1 (2.8)
Absolute change at Week 48, mean (SD)	-0.5 (2.0)	-0.3 (1.9)
Difference vs Placebo, mean (95% CI)	-0.2 (-1.4, 1.0)	
Perfusion Abnormalities		
Baseline, mean (SD)	4.0 (2.8)	4.3 (2.4)
Week 48, mean (SD)	3.3 (2.7)	4.8 (2.2)
Absolute change at Week 48, mean (SD)	-0.7 (2.5)	0.5 (2.6)
Difference vs Placebo, mean (95% CI)	-1.2 (-2.7, 0.4)	
Special Findings		
Baseline, mean (SD)	0.9 (1.3)	1.4 (1.5)
Week 48, mean (SD)	0.7 (1.1)	1.2 (1.7)
Absolute change at Week 48, mean (SD)	-0.2 (0.7)	-0.2 (1.2)
Difference vs Placebo, mean (95% CI)	0.0 (-0.5, 0.6)	

*Baseline was defined as the most recent non-missing measurement (scheduled or unscheduled) collected before the first dose of study drug.

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Table E5. Positive microbiology culture results*

n (%)	Lumacaftor/ivacaftor n=35		Placebo n=16	
	Baseline[†,‡]	Week 48[§]	Baseline[†]	Week 48
Methicillin-susceptible *Staphylococcus aureus*	15 (44.1)	13 (41.9)	8 (50.0)	12 (75.0)
Pseudomonas aeruginosa				
Mucoid	1 (2.9)	1 (3.2)	0	0
Nonmucoid	2 (5.9)	1 (3.2)	1 (6.3)	0
Small-colony variant	1 (2.9)	0	1 (6.3)	0

* Percentages are based on the number of available observations per visit. [†] Baseline was defined as the most recent nonmissing measurement before the initial administration of study drug. [‡] n=34. [§] n=31.

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Table E6. Summary of treatment-emergent respiratory events

Children, n (%)	Lumacaftor/ivacaftor n=35	Placebo n=16
Any treatment-emergent respiratory event	2 (5.7)	3 (18.8)
Dyspnea	1 (2.9)	2 (12.5)
Bronchospasm	1 (2.9)	0
Asthma	0	0
Bronchial hyperreactivity	0	0
Chest discomfort	0	0
Respiration abnormal	0	0
Wheezing	0	1 (6.3)
By maximum severity		
Mild	1 (2.9)	2 (12.5)
Moderate	1 (2.9)	1 (6.3)
Severe	0	0
Life threatening	0	0
Serious	0	0
Leading to treatment interruption	0	0
Leading to treatment discontinuation	0	0
Leading to death	0	0

Table E7. Summary of treatment-emergent elevated transaminase levels events and liver

function test results

Children, n (%)	Lumacaftor/ivacaftor n=35	Placebo n=16
ALT or AST level		
≤3 × ULN	30 (85.7)	16 (100.0)
>3 × ULN to ≤5 × ULN	0	0
>5 × ULN to ≤8 × ULN	4 (11.4)	0
>8 × ULN	1 (2.9)	0
ALT or AST and total bilirubin		
ALT or AST level >3 × ULN and total bilirubin level >2 × ULN	0	0
Any treatment-emergent elevated transaminase levels event*	3 (8.6)	0
ALT abnormal	0	0
ALT increased	3 (8.6)	0
AST abnormal	0	0
AST increased	2 (5.7)	0
By maximum severity		
Mild	2 (5.7)	0
Moderate	0	0
Severe	1 (2.9)	0
Life threatening	0	0

Serious	0	0
Leading to treatment interruption	1 (2.9)	0
Leading to treatment discontinuation	0	0

ALT, alanine aminotransferase; AST, aspartate aminotransferase; ULN, upper limit of normal. *

Includes children who experienced any of the treatment-emergent events shown in the subrows.

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References

E1. Eichinger M, Optazaite DE, Kopp-Schneider A, Hintze C, Biederer J, Niemann A, Mall MA, Wielpütz MO, Kauczor HU, Puderbach M. Morphologic and functional scoring of cystic fibrosis lung disease using MRI. *Eur J Radiol* 2012; 81: 1321-1329.

E2. Leutz-Schmidt P, Stahl M, Sommerburg O, Eichinger M, Puderbach MU, Schenk JP, Alrajab A, Triphan SMF, Kauczor HU, Mall MA, Wielpütz MO. Non-contrast enhanced magnetic resonance imaging detects mosaic signal intensity in early cystic fibrosis lung disease. *Eur J Radiol* 2018; 101: 178-183.

E3. Schwartz GJ, Munoz A, Schneider MF, Mak RH, Kaskel F, Warady BA, Furth SL. New equations to estimate GFR in children with CKD. *J Am Soc Nephrol* 2009; 20: 629-637.