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Original Article

Lumacaftor/ivacaftor in people with cystic fibrosis with an *A455E–CFTR* mutation

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ABSTRACT

Background: Previous in vitro organoid data showed *A455E–CFTR*, a rare *CFTR* mutation with 4.1% prevalence in the Netherlands, responds to lumacaftor/ivacaftor (LUM/IVA). We explored LUM/IVA's clinical efficacy in people with CF and ≥1 *A455E–CFTR* mutation.

Methods: Participants aged ≥12 years were randomized to 1 of 2 treatment sequences (LUM/IVA→placebo or placebo→LUM/IVA) with an 8–week washout period between. Primary endpoint was absolute change in ppFEV$_1$ from study baseline through 8 weeks. Additional endpoints were change in sweat chloride concentration (SwCl) and CFQ–R respiratory domain score. Correlations between organoid–based measurements and clinical endpoints were investigated.

Results: Twenty participants were randomized at 2 sites in the Netherlands. Mean absolute change in ppFEV$_1$ from study baseline through Week 8 showed a treatment difference of 0.1 percentage points (95% CI, −2.5 to 2.7; $P = 0.928$) between LUM/IVA (within–group mean change, 2.7) and placebo (within–group mean change, 2.6). The mean absolute change in SwCl concentration from study baseline through Week 8 showed a treatment difference of −7.8 mmol/L between LUM/IVA and placebo ($P = 0.004$), while the absolute change in CFQ–R respiratory domain score showed a treatment difference of 3.5 between LUM/IVA and placebo ($P = 0.469$). The in vitro organoid–based assay demonstrated a concentration–dependent swelling increase with LUM/IVA. Exploratory correlation analyses between organoid swelling and ppFEV$_1$ and SwCl outcomes showed correlation coefficients of 0.49 and −0.11, respectively.

Conclusions: In this exploratory study, LUM/IVA elicited an in vitro response in organoid swelling and in vivo response in SwCl in participants with CF and ≥1 *A455E–CFTR* mutation. The primary endpoint (ppFEV$_1$) did not show a statistically significant difference between LUM/IVA and placebo; correlations between in vitro and in vivo responses were not established (NCT03061331).

1. Introduction

Cystic fibrosis (CF) results from mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene that reduce the quantity and/or function of the CFTR protein, which regulates chloride transport across epithelia in exocrine organs, in-

Abbreviations: AE, adverse event; AUC, area under the curve; CF, cystic fibrosis; CFQ–R, Cystic Fibrosis Questionnaire–Revised; CFTR, cystic fibrosis transmembrane conductance regulator; FEV$_1$, forced expiratory volume in 1 s; ICH GCP, International Council for Harmonisation Good Clinical Practice; IVA, ivacaftor; LS, least squares; LUM, lumacaftor; LUM/IVA, lumacaftor/ivacaftor combination; MMRM, mixed–effects model for repeated measures; P, placebo; ppFEV$_1$, percent predicted forced expiratory volume in 1 s; SwCl, sweat chloride concentration.

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cluding the lung and pancreas [1]. Progressive lung function decline is the leading cause of mortality among people with CF (pwCF) [2,3].

p.Ala455Glu (*A455E*) is a class V mutation that generates CFTR protein with a shortened half–life, resulting in a reduction of mature CFTR protein [4–6]; in vitro studies suggest that the quantity of functional protein at the cell surface is 12% of wild type [7]. With this amount of functional protein, *A455E–CFTR* is considered a residual function mutation. Worldwide, *A455E* mutations have been reported in <0.1% of pwCF, although the prevalence varies by region [8,9]; in the Netherlands, the *A455E* mutation occurs in 4.1% of pwCF [9,10]. Clinical experience with the *A455E* mutation, initially associated with a less–severe CF phenotype, has shown differences in disease severity by early adulthood, with a range of lung function loss [8,11,12]. Although people with residual function mutations such as *A455E* develop clinical characteristics of CF more slowly than those homozygous for *F508del*, it progresses more rapidly in adolescents and young adults [13].

Ivacaftor (IVA) is a small–molecule CFTR potentiator that increases the channel open probability of CFTR at the cell surface [14]. Lumacaftor (LUM) and tezacaftor are small–molecule CFTR correctors that increase the quantity of CFTR delivered to the cell surface; these are combined with a CFTR potentiator, such as IVA, for their additive effects [15,16]. IVA has been approved (as of 2017) in the United States for treating people with an *A455E* mutation [14], and the combination of IVA and tezacaftor has been approved (as of 2018) in the United States [16] and European Union [17]. In the European Union, combination IVA and tezacaftor treatment is indicated for pwCF with an *A455E–CFTR* mutation who also have an *F508del–CFTR* mutation [17]. Combined lumacaftor/ivacaftor (LUM/IVA) therapy improves lung function and provides multisystemic clinical benefits in pwCF who are homozygous for the *F508del* mutation, a mutation that results in processing and trafficking defects [18]. Improvements in forced expiratory volume in 1 s (FEV_1) were observed as early as Day 15 in participants ≥12 years of age on LUM/IVA compared with those on placebo and were sustained through 24 weeks of treatment in the pivotal Phase 3 studies TRAFFIC and TRANSPORT [18]. Additional studies of LUM/IVA have led to approval of its use in pwCF as young as 2 years who are homozygous for the *F508del* mutation [15,19].

In vitro responses to CFTR modulators have previously been studied using Fischer rat thyroid or human bronchial epithelial cell systems [20,21]. A study in human bronchial epithelial cultures from pwCF homozygous for the *F508del* mutation showed that LUM enhanced forskolin–stimulated chloride and fluid transport; the addition of IVA increased this response [22]. More recently, a novel CFTR functional assay using cultures of intestinal stem cells, referred to as organoids, was developed [23]. Briefly, organoids derived from the intestinal stem cells of healthy controls swell in response to forskolin–induced activation of CFTR–dependent chloride secretion. Forskolin–induced swelling (FIS) is reduced in organoids derived from pwCF homozygous for the *F508del* mutation compared with those from healthy controls and could be restored by incubation of the organoids with LUM/IVA. LUM/IVA–induced improvement of organoid swelling was also observed in *A455E/F508del* organoids [24]. These in vitro data suggest that correction and potentiation by LUM/IVA may improve CFTR function in people with *A455E–CFTR* mutations.

Based on these preclinical data, we designed this study to explore the efficacy and in vitro responses of LUM/IVA in pwCF who had ≥1 *A455E–CFTR* mutation.

2. Methods

2.1. Clinical study design and participants

This exploratory, randomized, double–blind, placebo–controlled, multicenter, Phase 2 crossover study took place in the Netherlands (VX15–809–111; NCT03061331). It included two 8–week treatment periods (±7 days) separated by an 8–week (±7 days) washout period (Fig. 1A). Treatment Period 1 was from Day 1 to Week 8, and Treatment Period 2 was from Week 16 to Week 24. Participants were randomized 1:1 to receive the 2 treatment sequences. In Treatment Sequence 1, participants received LUM/IVA in Treatment Period 1 and placebo in Treatment Period 2 (LUM/IVA→P). In Treatment Sequence 2, participants received placebo in Treatment Period 1 and LUM/IVA in Treatment Period 2 (P→LUM/IVA). The approved dose of LUM/IVA (LUM 400 mg/IVA 250 mg every 12 h [q12h]) or matching placebo q12h was given orally. An 8–week washout period between the 2 treatment periods was chosen based on the terminal half–lives of LUM (26 h) and IVA (12 h) and on previous clinical study results [14,15,19].

Given the limited participant population available, a crossover design was chosen that enabled treatment of the same participant with both placebo and LUM/IVA in different treatment periods. The use of a double–blind design reduced the chance of bias. Participants with stable CF who were ≥12 years of age with ≥1 *A455E–CFTR* mutation and a percent predicted FEV_1 s ($ppFEV_1$) of ≥30% and ≤90% were eligible. This study was conducted in accordance with the International Council for Harmonisation Good Clinical Practice (ICH GCP) guidelines, consistent with the principles of the Declaration of Helsinki. Study documentation was approved by institutional ethics committees for each study site. All participants (and/or their legal guardians) provided written informed consent.

2.2. Objective and outcomes

Clinical and in vitro responses to LUM/IVA in participants ≥12 years of age with CF with the *A455E–CFTR* mutation were investigated. The primary endpoint was absolute change in $ppFEV_1$ from study baseline through 8 weeks of treatment of either treatment period, calculated using the 2012 Global Lung Initiative equations [25]. Other endpoints included absolute change in sweat chloride concentration from study baseline through 8 weeks of treatment and absolute change in Cystic Fibrosis Questionnaire–Revised (CFQ–R) respiratory domain score from study baseline at the end of 8 weeks of treatment in either period.

All treatment–emergent adverse events (AEs; defined as AEs that increased in severity or were newly developed at or after the initial dose of study drug in a given treatment period to 28 days after the last dose of study drug in that treatment period [or safety follow–up visit, whichever was last]) were assessed, documented, and reported in accordance with ICH GCP guidelines.

2.3. Statistical analysis

Because the *A455E–CFTR* mutation is so rare, no formal sample size calculations were conducted for this exploratory study. The planned sample size of 20 participants was based on the number of pwCF expected to be available and willing to participate. Assuming an estimated SD of the paired differences of 8.00 in $ppFEV_1$, the available sample size of 20 participants produces a 2–sided 95% CI for the mean treatment difference, with a precision (margin of error) of 3.74 percentage points.

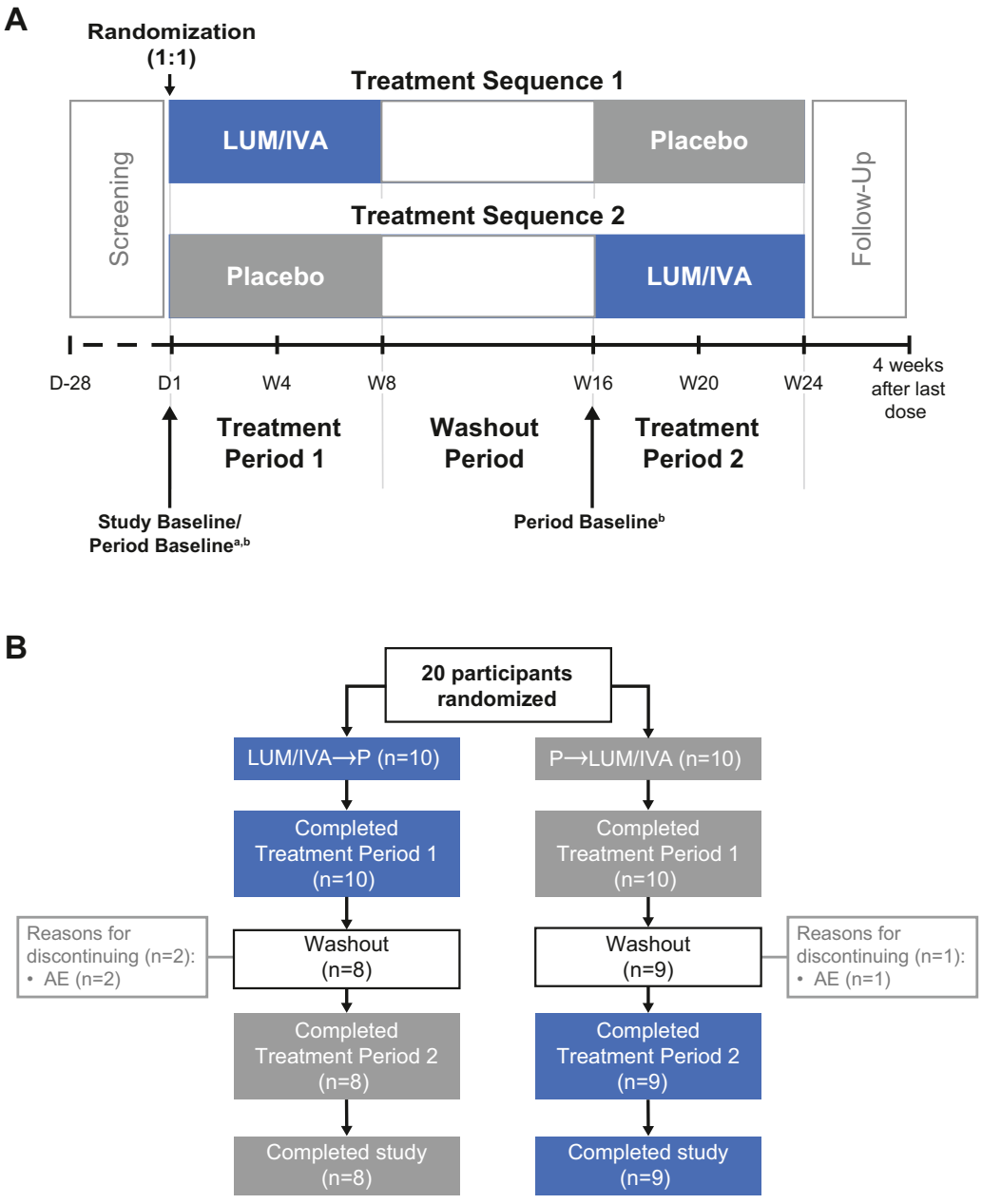


Fig. 1. Study Design and Participant Disposition. A. In this Phase 2, double–blind, placebo–controlled, crossover study, eligible participants were randomized (1:1) to 1 of 2 treatment sequences (LUM/IVA followed by placebo [Treatment Sequence 1] or placebo followed by LUM/IVA [Treatment Sequence 2]), consisting of two 8–week treatment periods separated by an 8–week washout period. B. Overall, 20 participants were randomized; all received ≥1 dose of study drug and completed Treatment Period 1. Of 10 participants randomized to Treatment Sequence 1, eight completed both treatment periods, and two discontinued treatment during the washout period due to AEs. Of 10 participants randomized to treatment sequence 2, nine completed both treatment periods, and one discontinued treatment during the washout period due to AEs. AE, adverse event; D, day; IVA, ivacaftor; LUM, lumacaftor; P, placebo; W, week. [a] Study baseline was the most recent nonmissing measurement (scheduled or unscheduled) collected prior to the first dose of study drug (either placebo or LUM/IVA) in the study. [b] Period baseline was the most recent nonmissing measurement (scheduled or unscheduled) collected before the first dose of study drug in Treatment Period 1 or Treatment Period 2.

For this crossover study, 2 different baselines were defined (Fig. 1A). *Study baseline* was defined as the most recent nonmissing measurement (scheduled or unscheduled) collected prior to the first dose of study drug (either placebo or LUM/IVA) in the study. The definition was applied to all demographics, background, and baseline characteristics and also to data analyses, including the primary endpoint analysis. *Period baseline* was defined as the most recent nonmissing measurement (scheduled or unscheduled) collected before the first dose of study drug in Treatment Period 1 or Treatment Period 2. Absolute changes from study baseline and period baseline were calculated as the postbaseline value minus the study baseline and period baseline value, respectively.

The primary analysis for the primary efficacy endpoint, the absolute change in ppFEV$_1$ from study baseline through 8 weeks of treatment of either treatment period, was based on a mixed–effects model for repeated measures (MMRM). The model included the absolute change from the study baseline in each treatment period as the dependent variable, with sequence, treatment, period, visit within period, and treatment–by–visit interaction as fixed effects; study baseline ppFEV$_1$ as a covariate; and participant nested

Table 1
Baseline participant demographics and characteristics.

	LUM/IVA→P (n = 10)	P→LUM/IVA (n = 10)	Overall (N = 20)
Female, *n* (%)	7 (70.0)	5 (50.0)	12 (60.0)
Age, mean (range), years	41.2 (14–-59)	34.7 (18–-51)	38.0 (14–-59)
≥12 years to <18 years, *n* (%)	2 (20.0)	0	2 (10.0)
≥18 years, *n* (%)	8 (80.0)	10 (100.0)	18 (90.0)
White, *n* (%)	10 (100.0)	10 (100.0)	20 (100.0)
Mutation genotype, *n* (%)			
A455E/F508del	9 (90.0)	9 (90.0)	18 (90.0)
A455E/other[a]	1 (10.0)	1 (10.0)	2 (10.0)
Weight, mean (SD), kg	64.8 (12.8)	72.6 (11.5)	68.7 (12.5)
Height, mean (SD), cm	170.3 (6.9)	178.2 (8.4)	174.3 (8.5)
BMI, mean (SD), kg/m^2	22.3 (3.5)	22.9 (3.5)	22.6 (3.4)
ppFEV$_1$, *n* (%)			
<40%	0	2 (20.0)	2 (10.0)
≥40% to <70%	9 (90.0)	5 (50.0)	14 (70.0)
≥70% to ≤90%	1 (10.0)	2 (20.0)	3 (15.0)
>90%	0	1 (10.0)	1 (5.0)
Mean ppFEV$_1$ (SD), percentage points	57.7 (9.4)	60.0 (20.0)	58.9 (15.3)
Mean sweat chloride concentration (SD), mmol/L	77.2 (12.3)	82.5 (7.5)	79.8 (10.3)
Mean CFQ–R respiratory domain score (SD)[b]	69.4 (15.3)	67.8 (13.8)	68.6 (14.2)
History of pancreatic insufficiency, *n* (%)	2 (20.0)	1 (10.0)	3 (15.0)

BMI, body mass index; CFQ–R, Cystic Fibrosis Questionnaire–Revised; LUM/IVA→P, participants receiving lumacaftor/ivacaftor in Treatment Period 1 followed by placebo in Treatment Period 2; P→LUM/IVA, participants receiving placebo in Treatment Period 1 followed by LUM/IVA in Treatment Period 2; ppFEV$_1$, percent predicted forced expiratory volume in 1 s.
[a] The 2 participants in the "other" mutation group had a class I *E60X* mutation.
[b] Data from the CFQ–R "Ages 12 and 13" and "Adolescents and Adults" versions were pooled for analysis.

within sequence as the random effect. In the model, visit was treated as a class variable. An unstructured covariance matrix was assumed for the repeated measurements of the same participant within each treatment period. Similar analyses were done for the other endpoints (sweat chloride concentration and CFQ–R respiratory domain score), with the baseline of the analyzed endpoint as the covariate. Differences between LUM/IVA and placebo endpoints through 8 weeks of treatment were obtained from the MMRM models, estimated by least–squares mean with a 2–sided 95% CI and a 2–sided *P* value. All reported *P* values for other endpoints are nominal *P* values. There was no control for multiplicity for this exploratory study. As a supportive sensitivity analysis, a prespecified MMRM analysis was conducted for the changes from period baseline in the primary endpoint ppFEV$_1$.

2.4. Participant–derived organoid–based measurements (FIS assay)

Participant–derived organoid responses to LUM/IVA and correlations to clinical outcomes (ppFEV$_1$, sweat chloride concentration) were also explored. Rectal biopsies were performed for individual participants during screening, and specimens were shipped to Hubrecht Organoid Technology, where intestinal crypts were isolated and expanded to establish organoid cultures. Organoid swelling was measured with an FIS assay using 42 different experimental conditions (Supplementary Material; Supplementary Table 1).

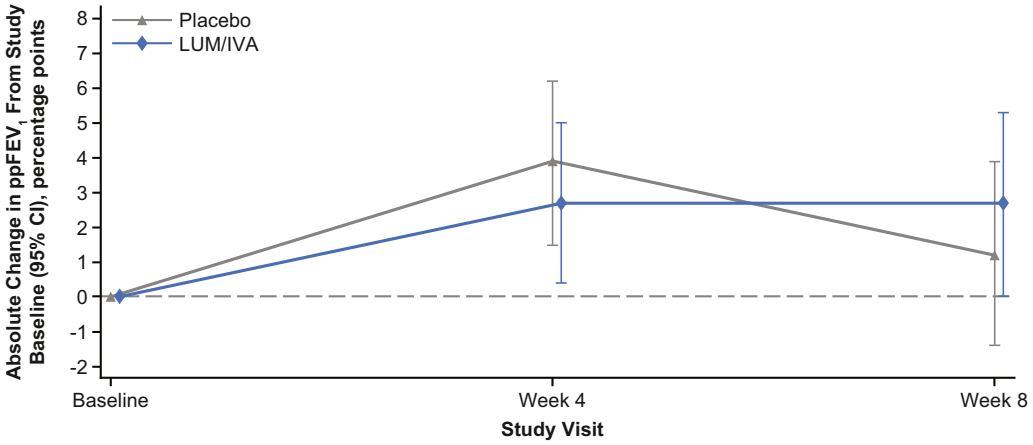
The background–corrected area under the curve (AUC) of organoid swelling at each experimental condition was summarized descriptively. Background–corrected swelling value refers to the difference between swelling of any nonzero LUM/IVA condition and that of the corresponding zero LUM/IVA condition at the same forskolin concentration. An exploratory correlation analysis between the in vitro organoid–based measurements and the responses to LUM/IVA treatment from period baseline in ppFEV$_1$ and sweat chloride concentration was conducted. The experimental conditions selected for the correlation analyses (forskolin, 0.128 µM; LUM, 3 µM; IVA, 3 µM) showed a large differentiation to forskolin alone and have previously shown correlation of organoid swelling response with a population–level clinical response [24]. Given the small sample size, Spearman rank correlation was used in the correlation analysis.

3. Results

Twenty participants were randomized 1:1 to receive the 2 treatment sequences at the 2 study sites. After randomization, participants continued their concomitant medications, most commonly for CF management (e.g., salbutamol, dornase alfa, and azithromycin).

Overall, 60% of participants were female, and the mean age was 38 years, with the majority (90%) being ≥18 years of age (Table 1). Ninety percent (18 of 20) of participants had an *F508del–CFTR* mutation on the second allele; the rest had *E60X–CFTR* on the second allele. Overall, the mean ppFEV$_1$ was 58.9 percentage points (range, 31.3 to 94.9) at baseline. All 20 randomized participants received ≥1 dose of study drug and were included in both the full analysis set and the safety set. All participants completed the 8 weeks of dosing in Treatment Period 1, and 17 (85%) completed the 8 weeks of dosing in Treatment Period 2 (Fig. 1B). Three participants discontinued the study during the washout period due to AEs. All 3 AEs were infective pulmonary exacerbations of CF that occurred outside the treatment–emergent period, were mild or moderate in severity, and deemed unrelated to the study drug. No participant discontinued during either treatment period.

The estimated mean absolute change in ppFEV$_1$ from study baseline through 8 weeks of treatment (primary endpoint) showed a treatment difference of 0.1 percentage points (95% CI, –2.5 to 2.7; $P = 0.928$) between LUM/IVA and placebo (least–squares absolute mean change: LUM/IVA, 2.7 percentage points [SE, 1.1]; placebo, 2.6 percentage points [SE, 1.2]; Fig. 2). In the prespecified supportive analysis, the estimated mean within–group absolute change in ppFEV$_1$ from period baseline through 8 weeks was 3.2 percentage points (SE, 1.0) with LUM/IVA and 1.1 percentage points (SE, 1.0) with placebo, which resulted in a treatment difference of 2.1 percentage points (95% CI, –0.6 to 4.8; $P = 0.117$; Table 2). The change in ppFEV$_1$ from baseline was further assessed for both Treatment Period 1, which was not subject to the impact of treatment crossover, and Treatment Period 2 (Supplementary Table 2).



	Placebo (n=18)	LUM/IVA (n=19)
Baseline $ppFEV_1$, mean (SD), percentage points	59.4 (15.9)	57.6 (14.6)
Absolute change in $ppFEV_1$ through Week 8, LS mean (95% CI), percentage points	2.6 (0.2 to 4.9)	2.7 (0.3 to 5.0)
P value within treatment	0.034	0.027
Difference, LS mean (95% CI), percentage points	0.1 (-2.5 to 2.7)	
P value vs placebo	0.928	

Fig. 2. Absolute Change in $ppFEV_1$ From *Study Baseline* Through Week 8 of Treatment. All participants received LUM 400 mg/IVA 250 mg every 12 h (blue line/diamonds) for 8 weeks and placebo (gray line/triangles) for 8 weeks according to 1 of 2 treatment sequences (LUM/IVA→placebo or placebo→LUM/IVA) with an 8–week washout period. Absolute change is expressed as LS mean (95% CI). IVA, ivacaftor; LS, least squares; LUM, lumacaftor; $ppFEV_1$, percent predicted forced expiratory volume in 1 s. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 2
Absolute change from period baseline in $ppFEV_1$ through Week 8.

	Placebo (n = 18)	LUM/IVA (n = 19)
Period baseline $ppFEV_1$, mean (SD), percentage points	60.6 (15.6)	56.6 (13.7)
Absolute change from period baseline through Week 8		
LS mean (95% CI)	1.1 (−1.0 to 3.3)	3.2 (1.1 to 5.4)
P value within treatment	0.291	0.005
LS mean difference (95% CI)	2.1 (−0.6 to 4.8)	
P value vs placebo	0.117	

IVA, ivacaftor; LS, least squares; LUM, lumacaftor; $ppFEV_1$, percent predicted forced expiratory volume in 1 s.

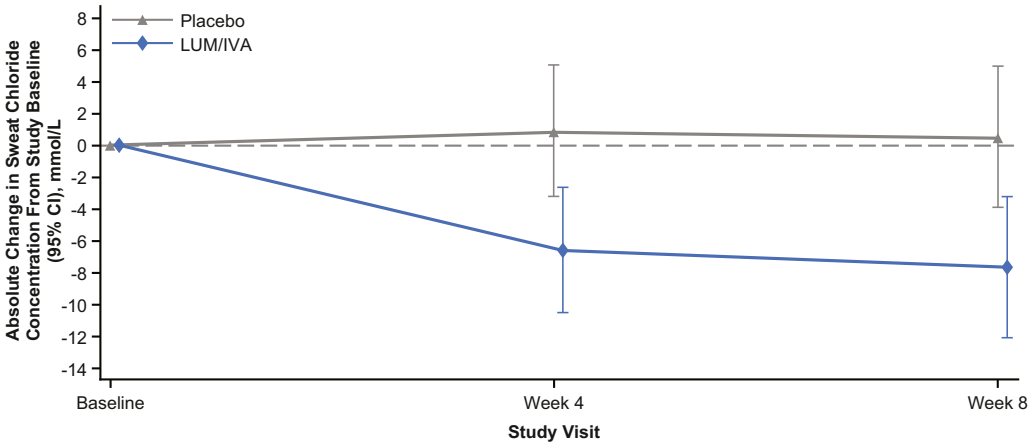
The mean absolute change in sweat chloride concentration from study baseline through Week 8 showed a treatment difference of −7.8 mmol/L (95% CI, −12.6 to −3.1; nominal $P = 0.004$) between the LUM/IVA group and the placebo group (Fig. 3). Changes of −7.1 mmol/L (SE, 1.7) in the LUM/IVA group and 0.7 mmol/L (SE, 1.8) in the placebo group were observed.

The mean absolute change in CFQ–R respiratory domain score from study baseline to the end of Week 8 showed a treatment difference of 3.5 points (95% CI, −6.4 to 13.4; nominal $P = 0.469$) between the LUM/IVA group and the placebo group. Changes of 6.4 points (SE, 3.9) in the LUM/IVA group and 2.9 points (SE, 4.0) in the placebo group were observed.

Administration of LUM/IVA in this CF population for approximately 8 weeks was generally safe and well tolerated. No participants had serious AEs or AEs that led to treatment discontinuation or interruption during the treatment period. The safety results were consistent with the known safety profile of LUM/IVA [18,26].

Of the 20 participants enrolled in the study, organoid cultures were successfully established for 16: Fourteen participants with the *A455E/F508del* genotype and 2 participants with the *A455E/E60X* genotype had organoid data. The descriptive mean estimates of background–corrected AUC of organoid swelling with each experimental condition (based on the concentrations of forskolin and LUM/IVA) are presented in Supplementary Figure 1.

The results of the in vitro organoid–based assay demonstrated a concentration–dependent increase in background–corrected AUC of swelling with LUM/IVA treatment. The background–corrected swelling response (i.e., AUC) was maximal and best differentiated at the forskolin 0.128–μM concentration and saturated at or above the LUM 3 μM/IVA 3 μM concentrations (Supplementary Figure 1). At this selected condition (forskolin 0.128 μM and LUM 3 μM/IVA 3 μM), the Spearman rank correlation coefficients between organoid AUC and the changes in $ppFEV_1$ and sweat chloride concentration observed with LUM/IVA treatment were 0.49 (n = 14; $P = 0.078$; Supplementary Figure 2) and −0.11 (n = 15; $P = 0.685$; Supplementary Figure 3), respectively.



	Placebo (n=18)	LUM/IVA (n=19)
Baseline sweat chloride concentration, mean (SD), mmol/L	81.6 (9.1)	80.1 (10.5)
Absolute change in sweat chloride concentration through Week 8, LS mean (95% CI), mmol/L	0.7 (-3.0 to 4.4)	-7.1 (-10.7 to -3.5)
P value within treatment	0.688	0.0004
Difference, LS mean (95% CI), mmol/L	-7.8 (-12.6 to -3.1)	
P value vs placebo	0.004	

Fig. 3. Absolute change from *Study Baseline* in sweat chloride concentration through Week 8 of treatment. All participants received LUM 400 mg/IVA 250 mg every 12 h (blue line/diamonds) or placebo (gray line//triangles) for 8 weeks according to 1 of 2 treatment sequences (LUM/IVA→placebo or placebo→LUM/IVA). Absolute change is expressed as LS mean (95% CI). IVA, ivacaftor; LS, least squares; LUM, lumacaftor. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

4. Discussion

Demonstrating the clinical efficacy of novel therapies targeting rare mutations or small participant populations is challenging. This exploratory study was conducted in a small cohort of pwCF with ≥1 *A455E–CFTR* mutation to evaluate the impact of LUM/IVA on clinical and in vitro endpoints.

The primary endpoint, absolute change in $ppFEV_1$ from study baseline through 8 weeks of treatment, did not show a significant treatment difference between the placebo and LUM/IVA groups. During this study, 2 participants had substantial increases in $ppFEV_1$ after 8 weeks of LUM/IVA treatment in Treatment Period 1, but their $ppFEV_1$ values did not return to study baseline level after the 8–week washout period. Given the study's small sample size, estimation of treatment effect based on the changes from study baseline can be impacted substantially by these 2 outlier participants due to the underlying assumption of equal baselines for Treatment Period 1 and Treatment Period 2. The prespecified supportive analysis of the changes in $ppFEV_1$ from period baseline does not depend on this assumption and showed a treatment difference of 2.1 percentage points between LUM/IVA and placebo.

Although the study failed to meet the primary endpoint, it is important to note that a treatment difference was observed between LUM/IVA and placebo in sweat chloride concentration. The overall efficacy results were suggestive of a clinical response with LUM/IVA treatment in pwCF with ≥1 *A455E* mutation. Potential long–term benefits, such as changes in the rates of pulmonary exacerbations, FEV_1 decline, and hospitalizations, were not evaluated in this study.

The rectal organoid FIS assay can be an effective strategy to identify rare *CFTR* mutations for CFTR modulator precision medicines. In the current study, a clear, concentration–dependent, in vitro organoid response to LUM/IVA was observed with participant–derived organoids, further suggesting that pwCF with the *A455E* mutation could be responsive to LUM/IVA.

Previous studies demonstrated that organoid swelling correlated with clinical changes in $ppFEV_1$ when participant outcomes were pooled from a heterogenous population and compared with preclinical in vitro results from different participants [24]. Moreover, Berkers et al recently published an analysis correlating in vitro organoid measurements with in vivo response of sweat chloride concentration and $ppFEV_1$ [27]—their results suggested that the organoid outcome was predictive of clinical outcome in individual participants. However, the current study could not demonstrate conclusive evidence regarding a correlation between the swelling of organoids and $ppFEV_1$ or sweat chloride response in pwCF with an *A455E–CFTR* mutation. The homogenous population of participants in this small study and the relatively small effects observed could have contributed to the results seen in this study.

Administration of LUM 400 mg/IVA 250 mg q12h for up to 8 weeks was safe and well tolerated in pwCF with the *A455E–CFTR* mutation. Safety results were consistent with those seen in other trials, and no new unexpected AEs were identified.

5. Conclusion

In this exploratory study, an in vitro response to LUM/IVA was observed in participant–derived organoids, and improvements in SwCl concentration were observed in pwCF treated with LUM/IVA compared to placebo. However, the primary clinical endpoint of absolute change in $ppFEV_1$ did not show a statistically significant difference between LUM/IVA and placebo, and correlations between in vitro and in vivo responses were not established.

Declaration of Competing Interest

All authors received nonfinancial support (assistance with manuscript preparation) from ArticulateScience LLC, which received funding from Vertex Pharmaceuticals Incorporated. Additional disclosures are as follows: JRD, ZY, and NK are employ-

ees of Vertex Pharmaceuticals Incorporated and may own stock or stock options in Vertex Pharmaceuticals Incorporated; PA was employed by Vertex Pharmaceuticals Incorporated at the time the study was conducted; HH reports personal fees from Gilead, PTC, Teva, and Vertex Pharmaceuticals Incorporated, and clinical trials with AbbVie and Vertex Pharmaceuticals Incorporated; JMB reports grants from Eloxx and Proteostasis, travel support from Proteostasis and Vertex Pharmaceuticals Incorporated, and royalties from the Royal Netherlands Academy of Sciences and Arts; RGJV is the CEO of Hubrecht Organoid Technology, a company based on the commercial implementation of the organoid technology, and reports grants and advisory board membership from Vertex Pharmaceuticals Incorporated; CKvdE reports grants from Eloxx, Galapagos NV, Gilead, GSK, Nutricia, ProQR, Proteostasis, Teva, and Vertex Pharmaceuticals Incorporated, and a patent (10006904) with royalties paid. SFB does not have any other disclosures to report.

CRediT authorship contribution statement

Gitte Berkers: Conceptualization, Methodology, Investigation, Resources, Writing - review & editing, Project administration. **Renske van der Meer:** Conceptualization, Methodology, Investigation, Writing - review & editing, Supervision. **Harry Heijerman:** Conceptualization, Methodology, Resources, Writing - original draft, Writing - review & editing, Visualization. **Jeffrey M. Beekman:** Conceptualization, Methodology, Writing - review & editing. **Sylvia F. Boj:** Supervision, Project administration. **Robert G.J. Vries:** Conceptualization, Methodology, Writing - review & editing, Supervision. **Peter van Mourik:** Investigation, Resources, Writing - review & editing. **Jamie R. Doyle:** Conceptualization, Methodology, Writing - original draft, Writing - review & editing. **Paul Audhya:** Conceptualization, Methodology, Formal analysis, Investigation, Resources, Writing - original draft, Writing - review & editing, Visualization, Supervision, Project administration, Funding acquisition. **Zheng (Jason) Yuan:** Methodology, Formal analysis, Writing - review & editing. **Nils Kinnman:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing - original draft, Writing - review & editing, Visualization, Supervision, Project administration. **C. Kors van der Ent:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Writing - review & editing, Visualization, Supervision, Funding acquisition.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jcf.2020.11.007.

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