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Original Article

Aerosolized lancovutide in adolescents (≥12 years) and adults with cystic fibrosis – a randomized trial

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ABSTRACT

Background: Lancovutide activates a chloride channel (TMEM-16A) other than the cystic fibrosis (CF) transmembrane conductance regulator protein and could benefit CF patients.

Methods: In this randomized, multi-center, double-blind, placebo-controlled, parallel-group trial 161 patients ≥12 years with a confirmed diagnosis of CF were randomized to either placebo (saline) or active drug in 3 different dosing schemes of 2.5mg inhaled lancovutide (once daily, every other day or twice a week) for eight weeks. The primary endpoint was the change in the forced expiratory volume in 1 second (FEV1) percent predicted. Secondary endpoints included further lung function parameters (FEV1 (absolute), functional vital capacity percent predicted, forced expiratory flow percent predicted, pulse oximetry), quality of life assessment, pulmonary exacerbations, hospitalization due to pulmonary exacerbations, time to first pulmonary exacerbation, duration of anti-inflammatory, mucolytic or antibiotic treatment, and safety.

Results: There was no significant difference in the change in FEV1 percent predicted, quality of life, other lung function parameters, pulmonary exacerbations or requirement of additional treatment between groups. Overall, the inhalation of lancovutide was safe although a higher rate of adverse events, especially related to the respiratory system, occurred as compared to placebo.

Conclusions: Lancovutide did not improve FEV1 percent predicted when compared to placebo (NCT00671736).

1. Introduction

Mutations of the cystic fibrosis transmembrane conductance regulator (CFTR) gene lead to absence or dysfunction of the CFTR anion channel in epithelial membranes and cause the clinical manifestations of cystic fibrosis (CF). Over the last decade, highly effective modulators of CFTR function have been developed that may

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prove transformational for approximately 90% of people who have specific CFTR genotypes (i.e. F508del, gating and residual function mutations) [1,2]. The most recent development, the combination of elexacaftor, tezacaftor and ivacaftor, two complementary CFTR correctors and one CFTR potentiator, showed substantial clinical improvements in a phase III trial in CF patients homozygous for F508del mutations and has received market approval from the European Medicines Agency and the US Food and Drug Administration [3]. However, there is still an unmet need for people with CF who have mutations that are not targeted by current modulators, are intolerant or have a poor response to CFTR modulators, or live in a region with poor access to modulators [4,5]. Alternate therapeutic strategies are needed to address these populations.

One possible strategy is not to correct or potentiate CFTR function itself, but to compensate the defective CFTR by targeting alternative chloride channels such as the calcium activated chloride channel TMEM16A [6]. Activation of TMEM16A is assumed to benefit patients by inducing chloride efflux and consecutive fluid secretion [6]. Different modulators for TMEM16A are currently being developed, although its role as a therapeutic target is not fully clear [6]. Denufosol, a ligand for the P2Y2 receptor that induces the downstream activation of TMEM16A, failed to show beneficial effects in long-term studies [7].

Lancovutide is a polycyclic peptide derived from *Streptomyces cinnamoneous* [8]. Lancovutide interacts with cellular membranes and changes the activity of ion channels [8]. The hypothesized mechanism of action is based on an increase in intracellular calcium that activates TMEM16A and increases chloride efflux [9]. However, Oliynyk et al. showed that the therapeutic range for TMEM16A activation is very narrow (around 2µM or 8µg/mL) [8]. Their group and others reported that at higher concentrations lancovutide seems to have unspecific effects on ion channels and is thought to disrupt the cell membrane, which would cause an unspecific chloride efflux, which is not mediated by TMEM16A [8,10]. Of note, the maximal concentration in BALF cells was 800ng/mg and about twenty times higher than those in brush biopsies [11].

In two phase II studies involving CF patients, lancovutide was well tolerated and improved pulmonary function when administered for 5 and 28 days, respectively. [12,13]

The aim of this randomized, controlled trial was to evaluate the safety and efficacy of three different lancovutide treatment regimens versus placebo in a larger trial over a longer treatment period (8 weeks).

2. Materials and methods

This was a multicenter, randomized, double-blind, placebo-controlled, parallel group, dose-finding trial with three different dose regimens designed to evaluate the efficacy and safety of aerosolized lancovutide (a designated Orphan Medicinal Product) in adolescents (≥12 years of age) and adults with CF. The trial was performed from October 2007 to July 2009 at 29 centers in nine European countries (Table S1). The trial was registered at a public database (NCT00671736) and was approved by the national regulatory authority in each participating country and by the institutional review board at each trial center. All subjects and/or parents, who participated in this trial, gave their written informed consent. The full trial protocol may be requested from the corresponding author.

A detailed list of all in- and exclusion criteria is presented in the supplement (Table S2). In short, patients ≥12 years of age with a confirmed diagnosis of CF (genotype, positive sweat chloride value >60mEq/l), with stable pulmonary disease, but absence of acute infection or other pulmonary disease were included in this trial.

2.1. Trial procedures

After a two-week screening period, patients were randomly assigned to one of the four trial groups in a 1:1:1:1 ratio. The treatment period lasted for 8 weeks. One group received placebo (0.9% sodium chloride solution), the low dose group (BIW) received 2.5mg lancovutide twice a week, the intermediate dose group (EOD) every other day, and the high dose group daily. An independent statistician created the randomization list using a validated system that automated the random assignment to treatment groups according to a random permuted block scheme. This schedule linked sequential numbers assigned to patients to treatment codes allocated at random. The investigational products were labeled with the randomization numbers. Patients received vials labeled with the trial days 1-55 and filled with either 5ml of placebo or lancovutide. Placebo and lancovutide vials could not be distinguished on the basis of appearances and physical characteristics. Thus, patients, clinicians and laboratory staff were blinded throughout the whole trial. The trial drug or placebo was administered using the PARI Master and PARI LC plus nebulizer and compressor system. Each subject was trained in the handling of these devices, and 15-30 minutes before inhalation of the trial drug salbutamol was used by all participating subjects. The investigators checked the patients' adherence at every visit by asking the subjects about drug intake, checking the returned vials and the patients' diaries for records.

Lung function testing was performed according to ATS/ERS standards [14] and lung function parameters were reported as percentages of predicted (%pred) on the basis of norms reported by Knudson et al. [15]

The cystic fibrosis questionnaire in the revised version (CFQ-R), consisting of 14 dimensions in total, was used to assess the subjects' self-reported quality of life. [16] The sum score of the respiratory and the physical functioning dimensions, as well as the sum score of all dimensions were assessed at days 0, 14, 28, 42, and 55.

Safety measures included ECG, spirometry and oximetry and were performed at every visit (screening, days 0, 7, 14, 28, 42, 55, and follow up). Clinical laboratory analyses, urinalysis and pregnancy tests were performed at the screening visit, at days 0, 28, and 55, and during the follow up visit 4 weeks after day 55. Adverse events and changes in the concomitant medication were documented during each visit throughout the trial. The number and time of pulmonary exacerbations were noted (a definition of pulmonary exacerbations is presented in the supplement).

3. Endpoints

The primary efficacy endpoint was the absolute change of FEV1%pred during the treatment period. [17,18] Secondary efficacy endpoints included the respiratory symptoms and physical functioning dimensions of the CFQ-R, the sum score of CFQ-R including all dimensions, relative changes in forced vital capacity in percent predicted (FVC%pred), relative changes in forced expiratory flow 25%-75% of FVC in percent predicted (FEF25-75%pred), changes in absolute FEV1 values, number of pulmonary exacerbations, number of hospitalization days due to pulmonary exacerbations, duration of therapy needed to treat bronchial obstruction, infection or inflammation during study participation (mucolytics, antibiotics, anti-inflammatory drugs), time to first exacerbation, and changes in SpO_2. Subgroup analyses were predefined for patients with a FEV1%pred ≤/>85% at screening and patients </≥ 18 years of age.

3.1. Statistics and Sample Size

Demographic variables and baseline characteristics were analyzed using descriptive statistics.

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At the time of the initiation of the study, there was no precedent for an expected treatment effect for a drug modulating epithelial chloride secretion. The sample size was thus based on results from a prior trial investigating the effects of azithromycin in CF patients, which found a mean group difference of 6 ± 12% (mean ± standard deviation) improvement in FEV1%. [19] Based on a t-test model, n=86 per group would be required to show a difference of 6% in the FEV1%pred with a power of 90% and a one-sided α=0.025. The sample size could be reduced by n=40 per group, applying the directional global test of the Wei-Lachin procedure, which is a multivariate extension of the Wilcoxon-Mann-Whitney test. [20] Two-group comparisons were performed using the Wei-Lachin procedure which analyzes six points during the observation time simultaneously. Thus, in case of this study a mean value across the time points week 4, 6 and 8 was calculated and inserted in the procedure.

The primary endpoint FEV1%pred was assessed in the intention to treat population. The directional global test (test of stochastic alternatives) of the Wei-Lachin procedure was conducted. In short, all treatment groups were tested versus placebo, in a pre-specified order, starting with the daily group, followed by the EOD and the BIW groups. Additionally, the mean values across three time-points (week 4, 6, 8) were taken as a derived variable for a univariate analysis. Different models were tested within the framework of conventional tests with linear contrasts. The best model was then fitted using nonlinear regression. Additionally, in case of a significant test result, two-group comparisons were performed. The multiple level alpha of 0.025 (one-sided) for this test procedure was based on the ICH Biostatistics guideline E9 [21].

Secondary endpoints were analyzed using the same procedure or the Wilcoxon-Mann-Whitney test, as applicable. However, these tests were only performed in a descriptive manner. Safety criteria and adverse events were evaluated in a descriptive manner without adjustment for multiple level alpha. Inferential statistics were performed as appropriate (Fisher's Exact test, Wilcoxon-Mann-Whitney test, Kolmogorov-Smirnov test, etc.).

4. Results

4.1. Patients

Between October 16th 2007 and July 15th 2009 190 patients were screened of which 161 were eligible and randomized (Fig. 1).

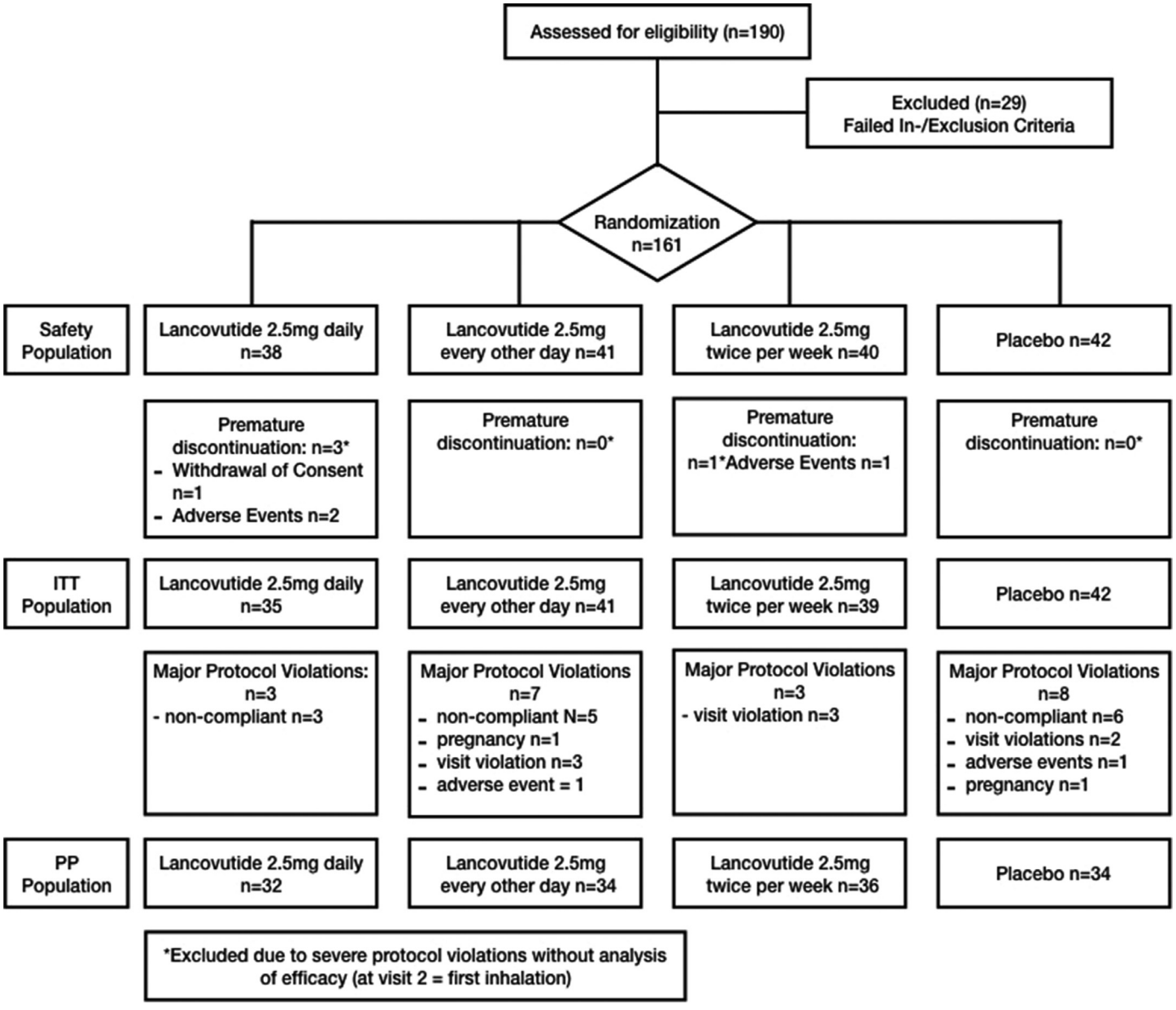

Fig. 1. Patient flow diagram.

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Table 1
Baseline demographics, medical history, concomitant medication, lung function parameters and quality of life.

	Lancovutide Daily	Lancovutide EOD	Lancovutide BIW	Placebo
Gender				
Male	16 (45.7%)	20 (48.9%)	20 (51.3%)	22 (52.4%)
Female	19 (54.3%)	21 (51.2%)	19 (48.7%)	20 (47.6%)
Age (years)	21 ± 2	22 ± 1	24 ± 2	20 ± 1
Height (cm)	163 ± 11	166 ± 10	166 ± 12	166 ± 7
Weight (kg)	53 ± 12	59 ± 12	59 ± 13	56 ± 9
BMI (kg/m²)	20 ± 2	21 ± 3	21 ± 3	20 ± 3
Medical history[$]				
No	22 (63%)	26 (63%)	22 (56%)	26 (62%)
Yes	13 (37%)	15 (37%)	17 (44%)	16 (38%)
Concomitant disease[#]				
No	22 (63%)	24 (58%)	23 (58%)	21 (50%)
Yes	13 (37%)	17 (42%)	16 (42%)	21 (50%)
Concomitant medication[§]				
No	4 (11%)	4 (10%)	2 (5%)	4 (10%)
Yes	31 (89%)	37 (90%)	37 (95%)	38 (90%)
Antimicrobials systemic	16 (42%)	20 (49%)	22 (55%)	25 (60%)
Antimicrobials inhalative	1 (3%)	10 (24%)	7 (18%)	7 (18%)
Inhalative	17 (45%)	14 (34%)	11 (28%)	16 (38%)
bronchodilatators or	10 (26%)	12 (24%)	10 (25%)	10 (24%)
corticosteroids	6 (16%)	4 (10%)	1 (3%)	4 (10%)
Dornase alpha	15 (39%)	15 (37%)	9 (23%)	11 (26%)
Sodium chloride inhalative	2 (5%)	2 (5%)	0	1 (2%)
Pancreatic enzymes	2 (5%)	1 (2%)	0	0
Insuline	15 (39%)	17 (41%)	17 (43%)	15 (36%)
Systemic steroids	12 (32%)	11 (27%)	8 (20%)	12 (29%)
Mucolytics	7 (18%)	10 (24%)	7 (18%)	6 (14%)
Vitamins				
Ursodeoxycholic acid				
FEV1 % pred.	69.1 ± 13.3[*]	74.1 ± 12.7	74.7 ± 14.5	75.0 ± 12.6
FEV1 absolute (l)	2.2 ± 0.5	2.5 ± 0.6	2.5 ± 0.7	2.5 ± 0.6
FVC % pred.	85.0 ± 14.0	89.6 ± 12.8	87.0 ± 12.4	86.2 ± 10.9
FEF25-75 % pred.	42.3 ± 19.6	49.7 ± 21.3	52.4 ± 23.4	53.4 ± 27.4
SpO_2 (%)	96.6 ± 1.7	97 ± 1.1	97 ± 1.3	96.8 ± 1.6
Sum score CFQ-R	897 ± 117	943 ± 105	882 ± 165	918 ± 112

FEV1 % pred.: Forced expiratory volume in 1 second in percent of the predicted value, FEV1 absolute: Forced expiratory volume in 1 second in liters, FVC % pred.: Forced vital capacity in percent of the predicted value, FEF25-75 % pred.: Forced expiratory flow 25%-75% of FVC in percent of the predicted value, SpO_2%: oxygen saturation measured by pulse oxymetry, Sum score CFQ-R: Sum score of cystic fibrosis quality of life questionnaire in the revised version.
Daily group: 2.5mg lancovutide daily,n=38 EOD group: 2.5mg lancovutide every other day, n=41 BIW group: 2.5mg lancovutide 2.5mg twice a week,n=40, placebo n=42

[$] Medical history is defined as any relevant disease other than CF associated conditions within the last 12 months.
[#] Concomitant disease is defined as any ongoing illness at the baseline
[§] Concomitant medication is defined as intake of any medication at the baseline, most frequent drugs listed
[*] $p<0.05$ vs. placebo group

In total, five patients discontinued the investigational product in the daily group (four patients due to an adverse event (AE), one due to withdrawal of consent), three in the EOD group (one due to an AE, one due to unwillingness to continue, and one due to pregnancy), one in the BIW group (due to an AE) and two in the placebo group (one due to an AE, one due to pregnancy). The trial ended after the last patient completed the follow-up visit on July 15th 2009. Table 1 presents baseline data. The groups were overall well balanced; only FEV1%pred was significantly lower in the daily group compared to the placebo group (p=0.049).

4.2. Primary endpoint

The treatment groups were not superior to placebo with regard to changes in FEV1%pred (Fig. 2, Table S3). A predefined subgroup analysis investigating the efficacy of lancovutide in patients <18 years also showed no significant differences between treatment groups (total n=71, Fig. 2). Another predefined subgroup included patients with a baseline FEV1%pred of >85%. There were no differences between treatment groups with regards to the primary endpoint. However, the number of patients with FEV1%pred of >85% was limited (5-10 patients per group, due to the limited number of patients, no further data for this subgroup will be reported).

4.3. Secondary endpoints

There were no differences between groups for the respiratory symptoms and physical functioning dimensions of the CFQ-R (Fig. 3). The CFQ-R sum score improved in all groups, but a significant difference was not found between treatment groups (Fig. S2). A subgroup analysis in patients <18 years also showed no significant differences for the sum score and the physical and respiratory dimensions (Fig. 3).

There were no differences between the treatment groups regarding the secondary endpoints relative changes in FVC%pred, relative changes in FEF25-75%pred, changes in absolute FEV1, total number of pulmonary exacerbations and patients with at least one pulmonary exacerbation (daily: 11 of 38 patients in the safety population, EOD: 12 of 41 patients, BIW: 5 of 40 patients, placebo: 7 of 42 patients, Fig. S1), number of hospitalization days due to pulmonary exacerbations, cumulative dose and duration of therapy needed to treat bronchial obstruction, infection or inflammation, time to first exacerbation, and changes in SpO_2.There was no difference in newly prescribed antibiotics or anti-inflammatory drugs between groups (supplement: table S4).

Treatments with the active substance did not improve the time to first pulmonary exacerbation (supplement: Fig. 1). However,

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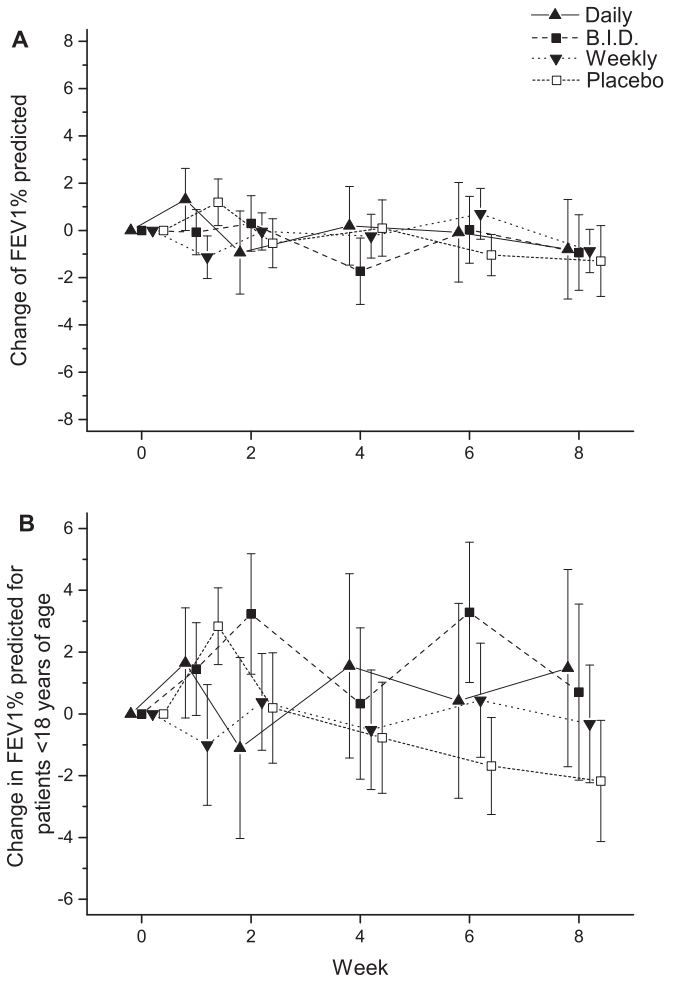

Fig. 2. Changes in FEV1 % predicted from baseline in all groups and all patients (panel A) and in all groups and patients younger than 18 years of age (predefined subgroup, panel B). Values represent means ± standard errors of the means. No statistical differences were found between treatment groups. Daily: 2.5mg lancovutide daily (n=35, ≤18 years n=17), EOD.: 2.5mg lancovutide every other day (n=41, ≤18 years n=18), BIW.: 2.5mg lancovutide twice a week (n=39, ≤18 years n=15), Placebo group (n=42, ≤18 years n=21).

only 5-10 patients per group suffered from pulmonary exacerbations.

4.4. Safety

Overall, significantly more AEs occurred in the three treatment groups (daily: 158; EOD: 219; BIW: 143) compared to the placebo group (113) (Table 2 and S5, S6). Likewise, AEs related to the trial drug were more frequently in the active groups (Table 2 and S5, S6). Noteworthy, no dose-dependency could be observed. The most frequent conditions involved cough, hemoptysis, lung disorders, headache and aggravated condition; an unspecific term relating to worsening of pre-existing symptoms/organ manifestations. Respiratory AEs are of special interest in this population. The number of respiratory AEs was significantly higher in the treatment groups, although again no dose-dependency could be observed.

Altogether four serious AEs occurred due to hospitalization, two in the EOD group, one in the BIW group and one in the placebo group (Table 2 and S5, S6): two patients developed hemoptysis, one a distal intestinal obstruction syndrome and one a pulmonary exacerbation.

Two pregnancies occurred, both children were born healthy and without complications.

5. Discussion

Up to now, there is only limited knowledge about the effects of lancovutide in humans. One phase I trial exposing six healthy subjects to lancovutide, and three phase II trials were conducted to investigate the safety and tolerability of lancovutide in CF patients [11–13]. The current phase II trial is the largest study including 119 patients exposed to lancovutide.

The current trial did not prove superiority of lancovutide over placebo, neither for the primary efficacy criterion change in FEV1%pred, nor for secondary endpoints of interest [17,18]. This contrasts previous phase II trials [12,13], which showed significant improvements in FEV1%pred, and thus motivated the design of the current trial. Noteworthy, in the first phase II trial, the high-dose group (lancovutide 2.5mg/d for only five consecutive days; n=6) had very good baseline lung function (median FEV1%pred 95.5%; FVC%pred 111.5%) [13]. In the second phase II trial, twelve patients ≥12 years of age and with FEV1%pred >60% received lancovutide 2.5mg/d for 28 days and, although not powered for that analysis, improvements in their lung function parameters compared to the placebo group were observed [12].

Overall, AEs occurred more frequently in the lancovutide groups compared to placebo, although no dose dependency was observed. Frequency and characteristics of adverse events were comparable to previous lancovutide trials [12,13] and other trials in CF patients [7,22]. Expectedly, AEs of respiratory/pulmonary origin occurred most frequently, which is in line with other CF trials [22].

The results of the current and earlier lancovutide trials [12,13] are somewhat comparable to the results of trials investigating the effects of denufosol, which initially showed promising results [23,24], but failed to meet the expectations in the confirmatory phase III trial [7]. Several reasons may explain the negative results of this trial. (i) The therapeutic range of lancovutide is narrow and the main effect being chloride efflux with consecutive fluid secretion into the airway system may be abolished at higher doses [6,8–10]. (ii) Lancovutide is an inhalative drug, which is naturally subject to great inter- and intra-patient variability, making correct drug dosing even more difficult. Furthermore, the distribution of any inhaled drug may vary substantially in different parts of the lungs and may decrease from central to more peripheral areas. (iii) The treatment period of 8 weeks may have been too short to detect positive effects of lancovutide on lung function parameters. EMA recently recommended that in confirmatory studies efficacy of new treatments regarding respiratory function should be evaluated after 12 months [17]. The estimated half-life of lancovutide was 25-91 days in BALF cells [11] and 64 days after pulmonary instillation in rats [25]. Thus, the treatment duration may have been too short to obtain true steady state conditions.

The exact mechanism of action of lancovutide is not fully clear. Experimental data argue against lancovutide being a specific inhibitor of TMEM16A and hint to a more unspecific mode of action, which, however, may alter TMEM16A activity [8,10]. But also the role of specific TMEM16A inhibition as a therapeutic option for CF patients has been questioned lately, which is emphasized by the simultaneous development of both, inhibitors and activators, of this ion channel [6]. TMEM16A is only minimally expressed in the airways of healthy humans, but is upregulated strongly by inflammatory stimuli. However, TMEM16A is primarily upregulated in mucus producing cells and only to a minor degree in ciliated epithelial cells and ionocytes, which are responsible for fluid secretion [26]. Thus, activation of TMEM16A may not induce a relevantly increased fluid secretion, but rather induce mucus secretion [6]. Moreover, TMEM16A may also contribute to bronchoconstriction by triggering contraction of airway smooth muscle cells [27]. A CFTR independent therapeutic approach clearly would be advantageous, because in contrast to available CFTR potentiat-

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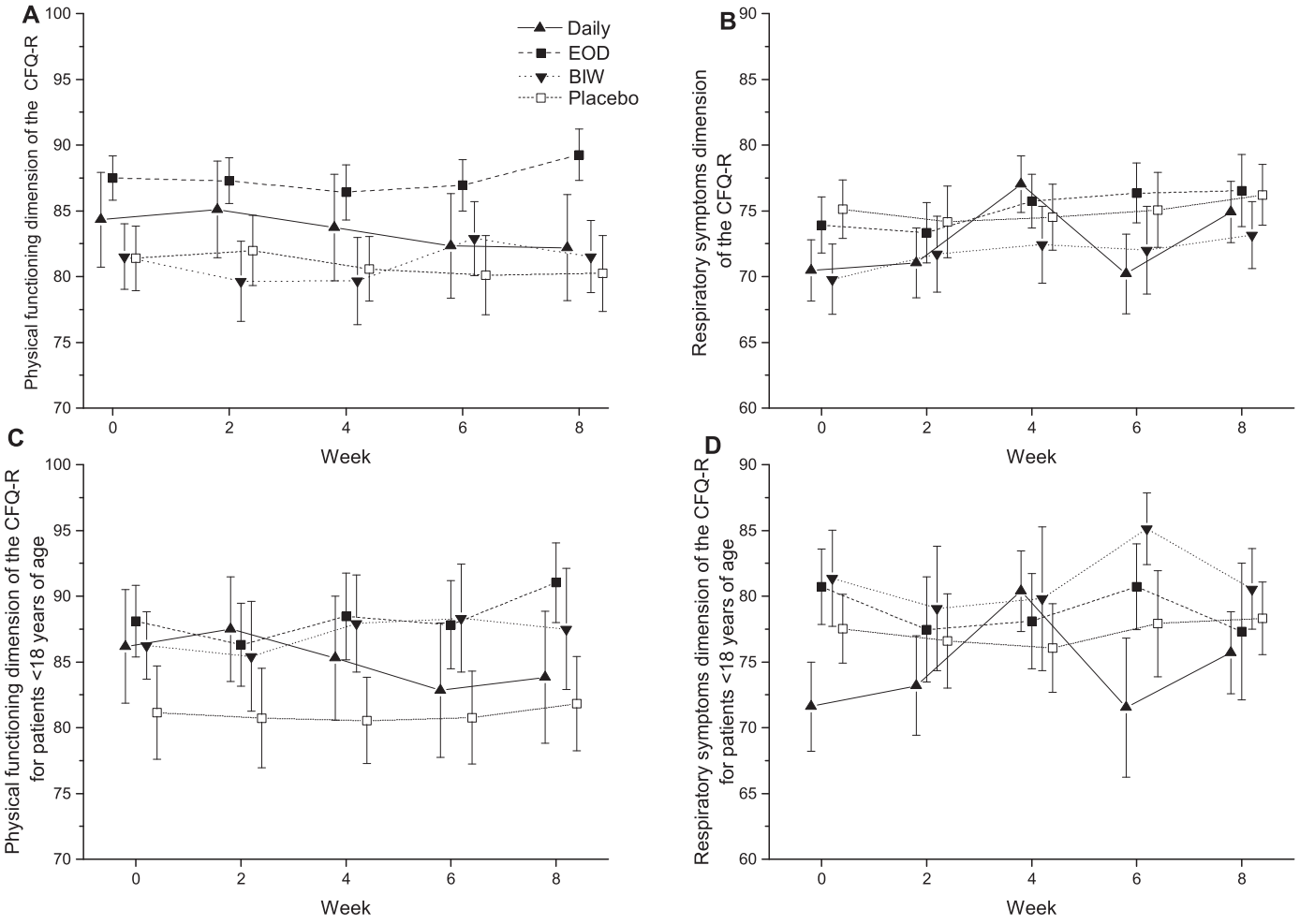

Fig. 3. Changes in the physical functioning dimension of the cystic fibrosis quality of life questionnaire (Panel A: all patients, Panel C: patients <18 years of age) and respiratory symptoms dimension (Panel B: all patients, Panel D: patients <18 years of age)) from baseline. Presented values represent means ± standard errors of the mean. No statistical differences were found between treatment groups. Daily: 2.5mg lancovutide daily (n=35, ≤18 years n=17), EOD.: 2.5mg lancovutide every other day (n=41, ≤18 years n=18), BIW.: 2.5mg lancovutide twice a week (n=39, ≤18 years n=15), Placebo group (n=42, ≤18 years n=21).

Table 2
Adverse events and their distribution over the groups.

	Lancovutide			
Event	Daily	EOD	BIW	Placebo
Any adverse event	158 (25.0%)	219 (34.6%)	143 (22.6%)	113 (17.9%)
Maximum Severity of adverse events				
Mild	118	165	93	58
Moderate	40	49	49	53
Severe	0	5	1	2
Life threatening	0	0	0	0
Serious adverse events	0	2	1	1
Adverse events leading to discontinuation of treatment	4	1	1	1
Adverse events leadting to death	0	0	0	0
Related adverse events	97 (26.7%)	104 (28.7%)	65 (17.9 %)	97 (26.7%)
Most common adverse events				
Cough	43	70	48	25
Hemoptysis	6	9	14	2
Lung disorder	6	8	3	3
Condition aggravated	11	9	3	3
Headache	12	7	2	4
Pyrexia	6	5	0	8
Nasopharyngitis	5	8	5	4
Rhinitis	4	3	4	9
Throat irritation	8	2	5	0

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ing/correcting therapies, all CF patients, regardless of the underlying CFTR mutation, could benefit. ETX001, a TMEM16A potentiator has recently shown promising results *in vitro* and in animal models improving mucociliary clearance and lung function without signs of TMEM16A-related side effects [28]. However, also other non-CFTR chloride channels, such as CLC-2, which is activated by lubiprostone, or SLC26A9, may provide interesting drug targets for CF patients [6,29].

Beside the mentioned limitations, it has to be noted that baseline FEV1%pred was lower in the daily group compared to other groups of the current trial, which may have biased against the efficacy and/or safety of lancovutide. Although this trial was the by far largest conducted investigating lancovutide in CF patients (and also large compared to other phase II trials in CF patients), the sample size in each group was still limited.

In conclusion, this trial could not show superiority of lancovutide over placebo. The lessons learned from the preclinical and clinical development program of lancovutide may inform others who are developing non-CFTR modulator strategies for people with CF.

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Declaration of Competing Interest

RW is founder and employee of AOP Orphan Pharmaceuticals AG. EE reports receiving consultancy fees from Vertex Pharmaceuticals, Chiesi Pharmaceuticals, Gilead Sciences, Vifor, and Insmed, and speaker's honoraria from Vertex Pharmaceuticals, Chiesi Pharmaceuticals, and Gilead Sciences. Dr. Ratjen reports grants and personal fees from Vertex, personal fees from Proteostasis, personal fees from Bayer, personal fees from TranslateBio, personal fees from Genentech, personal fees from Boehringer Ingelheim, personal fees from Calithera, outside the submitted work. All other authors report no relevant conflicts of interest regarding this work.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jcf.2020.08.014.

References

[1] Ramsey BW, Davies J, McElvaney NG, Tullis E, Bell SC, Drevinek P, et al. A CFTR potentiator in patients with cystic fibrosis and the G551D mutation. N Engl J Med 2011;365(18):1663–72.
[2] Wainwright CE, Elborn JS, Ramsey BW, Marigowda G, Huang X, Cipolli M, et al. Lumacaftor-ivacaftor in patients with cystic fibrosis homozygous for phe508del CFTR. N Engl J Med 2015;373(3):220–31.
[3] Heijerman HGM, McKone EF, Downey DG, Van Braeckel E, Rowe SM, Tullis E, et al. 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(10212):1940–8.
[4] da Silva Filho L, Zampoli M, Cohen-Cymberknoh M, Kabra SK. Cystic fibrosis in low and middle-income countries (LMIC): a view from four different regions of the world. Paediatr Respir Rev 2020.
[5] Gramegna A, Contarini M, Aliberti S, Casciaro R, Blasi F, Castellani C. From ivacaftor to triple combination: a systematic review of efficacy and safety of CFTR modulators in people with cystic fibrosis. Int J Mol Sci 2020;21(16).
[6] Kunzelmann K, Ousingsawat J, Cabrita I, Dousova T, Bahr A, Janda M, et al. TMEM16A in cystic fibrosis: activating or inhibiting? Front Pharmacol 2019;10:3.
[7] Ratjen F, Durham T, Navratil T, Schaberg A, Accurso FJ, Wainwright C, et al. Long term effects of denufosol tetrasodium in patients with cystic fibrosis. J Cyst Fibros 2012;11(6):539–49.
[8] Oliynyk I, Varelogianni G, Roomans GM, Johannesson M. Effect of duramycin on chloride transport and intracellular calcium concentration in cystic fibrosis and non-cystic fibrosis epithelia. APMIS 2010;118(12):982–90.
[9] Cloutier MM, Guernsey L, Sha'afi RI. Duramycin increases intracellular calcium in airway epithelium. Membr Biochem 1993;10(2):107–18.
[10] Zebedin E, Koenig X, Radenkovic M, Pankevych H, Todt H, Freissmuth M, et al. Effects of duramycin on cardiac voltage-gated ion channels. Naunyn Schmiedebergs Arch Pharmacol 2008;377(1):87–100.
[11] Steiner I, Errhalt P, Kubesch K, Hubner M, Holy M, Bauer M, et al. Pulmonary pharmacokinetics and safety of nebulized duramycin in healthy male volunteers. Naunyn Schmiedebergs Arch Pharmacol 2008;378(3):323–33.
[12] Eber E, Gyurkovics K, Eichler I, Thalhammer GH, Widmann R. Safety, tolerability and efficacy of a multiple-dose regimen of aerosolized Moli1901 in adolescents and adults with cystic fibrosis and mild to moderate lung disease. Pediatr Pulmonol 2007(Suppl. 30):301–2 Abstract 282.
[13] Grasemann H, Stehling F, Brunar H, Widmann R, Laliberte TW, Molina L, et al. Inhalation of Moli1901 in patients with cystic fibrosis. Chest 2007;131(5):1461–6.
[14] Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, et al. Standardisation of spirometry. Eur Respir J 2005;26(2):319–38.
[15] Knudson RJ, Burrows B, Lebowitz MD. The maximal expiratory flow-volume curve: its use in the detection of ventilatory abnormalities in a population study. Am Rev Respir Dis 1976;114(5):871–9.
[16] Goss CH, Quittner AL. Patient-reported outcomes in cystic fibrosis. Proceed Am Thorac Soc 2007;4(4):378–86.
[17] EMA. Guideline on the clinical development of medicinal products for the treatment of cystic fibrosis; 2009.
[18] Doring G, Elborn JS, Johannesson M, de Jonge H, Griese M, Smyth A, et al. Clinical trials in cystic fibrosis. J Cyst Fibros 2007;6(2):85–99.
[19] Saiman L, Marshall BC, Mayer-Hamblett N, Burns JL, Quittner AL, Cibene DA, et al. Azithromycin in patients with cystic fibrosis chronically infected with pseudomonas aeruginosa: a randomized controlled trial. JAMA: J Am Med Asso 2003;290(13):1749–56.
[20] Wei LJ, Lachin JM. Two-sample asymptotically distribution-free tests for incomplete multivariate observations. J Am Stat Asso 1984;79(387):653–61.
[21] ICH E-. E9 statistical principles for clinical trials - note for guidance on statistical principles for clinical trials (CPMP/ICH/363/96). 1998.
[22] Middleton PG, Mall MA, Drevinek P, Lands LC, McKone EF, Polineni D, et al. Elexacaftor-tezacaftor-ivacaftor for cystic fibrosis with a single Phe508del Allele. N Engl J Med 2019;381(19):1809–19.
[23] Deterding RR, Lavange LM, Engels JM, Mathews DW, Coquillette SJ, Brody AS, et al. Phase 2 randomized safety and efficacy trial of nebulized denufosol tetrasodium in cystic fibrosis. Am J Respir Crit Care Med 2007;176(4):362–9.
[24] Accurso FJ, Moss RB, Wilmott RW, Anbar RD, Schaberg AE, Durham TA, et al. Denufosol tetrasodium in patients with cystic fibrosis and normal to mildly impaired lung function. Am J Respir Crit Care Med 2011;183(5):627–34.
[25] McNulty MJ, Hutabarat RH, Findlay JW, Devereux K, Knick VC, Harvey RJ, et al. Pharmacokinetics and tissue distribution of the nonadecapeptide Moli1901 in rats and mice. Xenobiotica 2003;33(2):197–210.
[26] Benedetto R, Cabrita I, Schreiber R, Kunzelmann K. TMEM16A is indispensable for basal mucus secretion in airways and intestine. FASEB J 2019;33(3):4502–12.
[27] Centeio R, Cabrita I, Benedetto R, Talbi K, Ousingsawat J, Schreiber R, et al. Pharmacological inhibition and activation of the Ca(2+) activated Cl(-) channel TMEM16A. Int J Mol Sci 2020;21(7).
[28] Danahay HL, Lilley S, Fox R, Charlton H, Sabater J, Button B, et al. TMEM16A potentiation: a novel therapeutic approach for the treatment of cystic fibrosis. Am J Respir Crit Care Med 2020;201(8):946–54.
[29] MacDonald KD, McKenzie KR, Henderson MJ, Hawkins CE, Vij N, Zeitlin PL. Lubiprostone activates non-CFTR-dependent respiratory epithelial chloride secretion in cystic fibrosis mice. Am J Physiol Lung Cell Mol Physiol 2008;295(5):L933–40.