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Original Article

Riociguat for the treatment of Phe508del homozygous adults with cystic fibrosis

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A R T I C L E   I N F O

A B S T R A C T

Background: Riociguat is a first-in-class soluble guanylate cyclase stimulator for which preclinical data suggested improvements in cystic fibrosis transmembrane conductance regulator (CFTR) function.

Methods: This international, multicenter, two-part, Phase II study of riociguat enrolled adults with cystic fibrosis (CF) homozygous for Phe508del *CFTR*. Part 1 was a 28-day, randomized, double-blind, placebo-controlled study in participants not receiving CFTR modulator therapy. Twenty-one participants were randomized 1:2 to placebo or oral riociguat (0.5 mg three times daily [tid] for 14 days, increased to 1.0 mg tid for the subsequent 14 days). The primary and secondary efficacy endpoints were change in sweat chloride concentration and percent predicted forced expiratory volume in 1 second (ppFEV$_1$), respectively, from baseline to Day 14 and Day 28 with riociguat compared with placebo.

Results: Riociguat did not alter CFTR activity (change in sweat chloride) or lung function (change in ppFEV$_1$) at doses up to 1.0 mg tid after 28 days. The most common drug-related adverse event (AE) was headache occurring in three participants (21%); serious AEs occurred in one participant receiving riociguat

(7%) and one participant receiving placebo (14%). This safety profile was consistent with the underlying disease and the known safety of riociguat for its approved indications.

Conclusions: The Rio-CF study was terminated due to lack of efficacy and the changing landscape of CF therapeutic development. The current study , within its limits of a small sample size, did not provide evidence that riociguat could be a valid treatment option for CF.

Clinical trial registration number: NCT02170025.

Abbreviations

AE	adverse event
ATS	American Thoracic Society
AUC	area under the plasma concentration-time curve
$AUC(0–8h)_{ss}$	area under the plasma concentration-time curve over a dosing interval of 8 hours at steady-state
CONSORT	consolidated standards of reporting trials
CF	cystic fibrosis
CFTR	cystic fibrosis transmembrane conductance regulator
cGMP	cyclic guanosine monophosphate
C_{max}	maximum plasma concentration
CTEPH	chronic thromboembolic pulmonary hypertension
C_{trough}	plasma concentration at trough
ERS	European Respiratory Society
FDA	Food and Drug Administration
FEV_1	forced expiratory volume in 1 second
$LCI_{2.5}$	lung clearance index
MedDRA	Medical Dictionary for Regulatory Activities
NO	nitric oxide
PAH	pulmonary arterial hypertension
PDE5	phosphodiesterase type 5
ppFEV	percent predicted FEV_1
SAE	serious adverse event
SD	standard deviation
sGC	soluble guanylate cyclase
tid	three times daily
VSMC	vascular smooth muscle cell

1. Introduction

Cystic fibrosis (CF) is an autosomal recessive genetic disease caused by mutations of the *Cystic Fibrosis Transmembrane Conductance Regulator* (*CFTR*) gene. The most common CFTR mutation, Phe508del, occurs in approximately 90% of patients with CF, with approximately half being homozygous for this mutation [1]. The Phe508del mutation prevents the correct processing and folding of the CFTR protein, leading to CF symptoms [2]. CFTR modulators restore the function of the defective CFTR channel and could therefore be disease-modifying. At the initiation of this study, two disease-modifying treatments were approved: ivacaftor for patients with a gating mutation [3], and ivacaftor combined with lumacaftor [4] for patients homozygous for Phe508del. These modulators treat the basic defect in CF. Whilst ivacaftor has shown substantial effects in participants with a gating mutation, the impact of lumacaftor/ivacaftor on CFTR function and pulmonary outcomes in participants homozygous for Phe508del is modest [5]. Thus at the time of the study, there remained an unmet need for a highly effective CFTR modulator therapy for Phe508del [6].

Evidence suggests that accumulation of cyclic guanosine monophosphate (cGMP) can modulate CFTR activity [7]. The degradative action of cGMP-specific phosphodiesterase type 5 (PDE5) on cGMP in the vascular smooth muscle cells (VSMCs) can be blocked by PDE5 inhibitors (PDE5i) [8]. PDE5i increase CFTR function *in vitro* [9], reduce mucin secretion [10], and impact nasal potential difference [11,12] in Phe508del CF mouse models, suggesting that cGMP modulation may be a potential therapeutic treatment for patients with Phe508del CFTR. PDE5i and riociguat, a soluble guanylate cyclase (sGC) stimulator, act on different molecular targets in the same pathway. Riociguat elevates cGMP levels via stimulation of the nitric oxide (NO)-sGC-cGMP pathway [13]. It has a dual mode of action that stabilizes NO binding to sGC as well as directly stimulating sGC independent of NO leading to VSMC relaxation [13], and is approved for the treatment of adults with pulmonary arterial hypertension (PAH) and inoperable or persistent/recurrent chronic thromboembolic pulmonary hypertension (CTEPH) [14,15].

In vitro and *in vivo* data in transgenic mice with the Phe508del CFTR mutation suggested that sGC stimulators may act to restore CFTR function by enhancing processing of Phe508del CFTR and its functional expression on the cell surface (Supplementary data; Supplementary Figs. 1–6). Therefore, the Rio-CF study was designed to investigate the effect of riociguat in adults with CF homozygous for the Phe508del *CFTR* mutation. We report here the efficacy and safety results from the Rio-CF study Part 1.

2. Methods

2.1. Study design

Rio-CF was an international, multicenter, two-part, Phase II study of riociguat in adults with CF homozygous for Phe508del CFTR with mild-to-moderate pulmonary disease conducted from September 2014 to January 2017 (Clinicaltrials.gov identifier: NCT02170025) (Supplementary Fig. 7). Part 1 was a 28-day, randomized, double-blind, placebo-controlled study in adults with CF not receiving CFTR modulator therapy. Participants were randomized 1:2 to placebo or oral riociguat 0.5 mg orally three times daily (tid) for 14 days, increasing to 1.0 mg tid for the subsequent 14 days. Unpublished preclinical data were used for dose prediction (Bayer AG, data on file). Detailed information on groups, and the planned design of Part 2 can be found in the Supplementary methods.

The study was conducted in accordance with Good Clinical Practice and the Declaration of Helsinki, and was approved by the independent ethics committees or institutional review boards at participating centers. All participants provided written, informed consent.

2.2. Participants

Full inclusion and exclusion criteria are listed in the Supplementary methods. Potential participants were included if they were ≥18 years old with a confirmed diagnosis of CF and were homozygous for Phe508del. Other key inclusion criteria were forced expiratory volume in 1 second (FEV_1) between 40% and 100% of predicted at screening, stable lung disease with no ongoing or recent pulmonary exacerbation, and no change in respiratory treatment within the 4 weeks prior to screening.

2.3. Study endpoints and assessments

The primary efficacy endpoint in Part 1 was mean change from baseline to Day 14 and Day 28 in sweat chloride concentration in adults with CF receiving riociguat compared with placebo. The primary endpoint also included an assessment of safety and tolerability. The secondary endpoint in Part 1 was mean change from baseline to Day 14 and Day 28 in percent predicted FEV_1 ($ppFEV_1$). Exploratory endpoints included changes from baseline to Day 14 and Day 28 in nasal potential difference and lung clearance index ($LCI_{2.5}$). Efficacy assessments were also taken 14 days after the last treatment.

The collection of sweat samples was performed using the Food and Drug Administration (FDA)-approved Wescor Macroduct® device (ELITechGroup, Puteaux, France), with measurement of sweat chloride concentration at a central laboratory. Safety assessments included laboratory measurements and monitoring of adverse events (AEs) according to Medical Dictionary for Regulatory Activities (MedDRA) version 19.1, with symptomatic hypotension and hemoptysis defined as AEs of special interest. Nasal potential difference measurements were performed according to the joint Cystic Fibrosis Foundation-European Cystic Fibrosis Society standard operating procedure, with total CFTR-dependent chloride secretion as a measure of CFTR activity [16]. $LCI_{2.5}$ was measured from nitrogen multiple breath washouts with the Exhalyzer D (Ecomedics, Duernten, Switzerland). $LCI_{2.5}$ was calculated as the number of lung volume turnovers required to reach 2.5% of the starting nitrogen concentration; site training, certification, and central over-reading were described previously [17].

Sweat samples, nasal potential difference tracings, and $LCI_{2.5}$ recordings were sent to a central laboratory or reading facility for quality checking, analysis, and data interpretation. Measurement of $ppFEV_1$ was performed according to American Thoracic Society (ATS)/European Respiratory Society (ERS) guidelines using the Global Lung Function Initiative calculation as a reference [18,19]. Non-compliance was defined as any reported missed riociguat intake prior to the efficacy measurement time point and by respective pharmacokinetic data.

Riociguat plasma profiles were measured after the first 0.5 mg dose on the first treatment day and after the first day of up-titration to 1.0 mg on Day 15. Additionally, sparse steady-state pharmacokinetic samples were collected on the last day of administration of 0.5 mg (Day 14) and 1.0 mg (Day 28). Based on this observed data, riociguat exposure in participants with CF was assessed by estimation of the area under the plasma concentration-time curve over a dosing interval of 8 hours at steady-state ($AUC[0–8h]_{ss}$), the plasma concentration at trough (C_{trough}), and the maximum plasma concentration (C_{max}) at a dose of 0.5 mg tid or 1 mg tid using a previously published population pharmacokinetic model for riociguat in patients with PAH and CTEPH [20].

2.4. Statistical methods and populations analyzed

Planned enrollment was approximately 18 adults with CF to achieve a sample size of at least 12 evaluable adults with CF: eight in the riociguat group and four in the placebo group. The sample size was calculated to be sufficient for an exploratory assessment of the effect of riociguat on sweat chloride concentration (please see Supplementary methods for further details).

The safety analysis set included all participants who received at least one dose of riociguat. Efficacy was assessed in the pharmacodynamic analysis set. Efficacy analyses were adapted post hoc as a result of study termination after Part 1: a sensitivity analysis was conducted in which sweat chloride concentration measured 14 days after the last treatment was used as a reference for off-treatment measurements and as a surrogate for baseline. When

reporting these data, *treatment response* refers to the difference between the baseline value and subsequent outcomes in each of the placebo, riociguat 0.5 mg, and riociguat 1.0 mg groups, whereas *treatment effect* refers to the difference in outcomes between the active (riociguat 0.5 mg or 1.0 mg) and placebo groups.

All variables and outcomes were analyzed descriptively: categorical variables by frequency tables and continuous variables by summary statistics (mean, standard deviation [SD], median, minimum, and maximum). Further, primary efficacy analysis and sensitivity was performed using a Bayesian approach applying a hierarchical model to account for individual participant effects.

3. Results

3.1. Participants

Overall, 31 adults were screened from 10 centers across seven countries: Belgium, Canada, France, Germany, the Netherlands, the UK, and the USA. Of these, 21 were enrolled and randomized to treatment: 14 to riociguat and seven to placebo (Fig. 1). All participants randomized to riociguat received at least one dose. Two participants, both randomized to riociguat, discontinued the study due to AEs.

Baseline demographics were well balanced between the treatment arms (Table 1). Mean age was 28 years and 76% of participants were male. Seven participants in the riociguat arm and four in the placebo arm had major protocol deviations, mostly related to missed doses or non-compliance with the dosing schedule; the mean (SD) treatment durations were 26.0 (6.7) and 28.4 (0.5) days in the riociguat and placebo arms, respectively.

A comparison of pharmacokinetic parameters of riociguat exposure in plasma for participants with CF with that for patients with PAH in the PATENT-1 study [20], and healthy male subjects from a previous dose proportionality study [21] can be found in Supplementary Table 1. In line with the pharmacokinetic model predictions, after riociguat, the geometric mean of AUC and C_{max} was larger in participants with CF compared with healthy subjects, whereas the geometric mean and median of AUC and C_{trough} were smaller in participants with CF compared with patients with PAH from PATENT-1, but the ranges of the studies largely overlapped.

3.2. Riociguat efficacy

3.2.1. Primary endpoint

Sixteen of 21 participants (nine riociguat; seven placebo) were evaluable for efficacy analyses. Five riociguat-treated participants were non-evaluable due to missing sweat chloride data (n = 2), premature study discontinuation (n = 2), or non-compliance with the dosing schedule (n = 1).

At baseline, there was no difference between the treatment arms for sweat chloride concentration absolute values (mean [SD]: riociguat, 96.3 [17.3] mmol/L; placebo, 94.5 [12.8] mmol/L). During the study, escalating doses of riociguat up to 1.0 mg tid did not reduce sweat chloride concentration versus placebo. On Day 14, mean (SD) sweat chloride concentration values had increased from baseline to 103.4 (9.4) mmol/L with riociguat (n = 9) and 103.2 (16.3) mmol/L with placebo (n = 7), resulting in a treatment response change from baseline of +7.1 (10.3) mmol/L and +8.7 (8.2) mmol/L, respectively (Fig. 2a). On Day 28, the treatment response for change from baseline in sweat chloride concentration was +3.4 [11.0] mmol/L in the riociguat arm (n = 9) compared with +9.0 (12.7) mmol/L in the placebo arm (n = 7) (Fig. 2a). At follow-up (14 days after the last treatment dose), treatment response for change from baseline in sweat chloride concentration was +2.9 (10.6) mmol/L in the riociguat arm (n = 9) and +9.8 (12.5) mmol/L in the placebo arm (n = 6) (Fig. 2a).
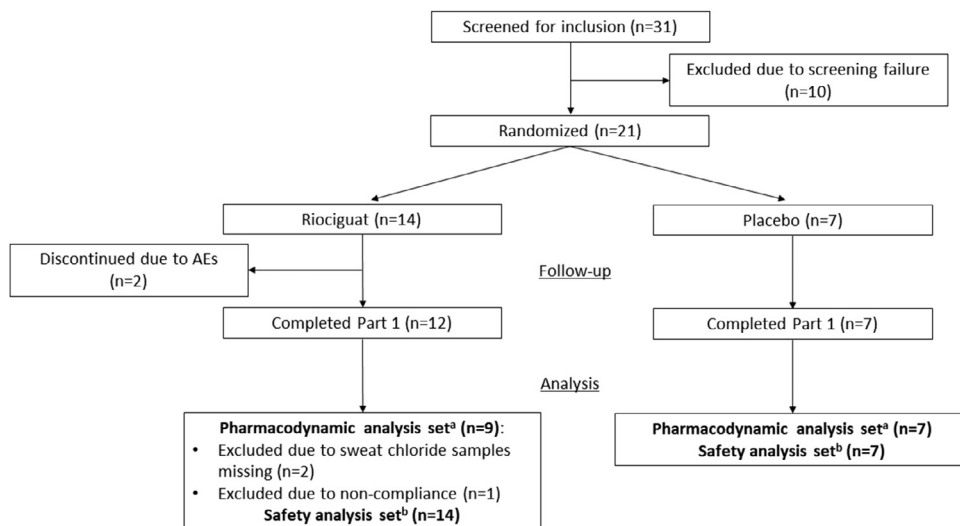
Fig. 1. CONSORT diagram of participant disposition.
[a] Pharmacodynamic analysis set included only participants randomized to a treatment who completed the study without circumstances affecting the validity of the efficacy analysis.
[b] Safety analysis set included all participants who received a dose of riociguat. AE, adverse event; CONSORT, Consolidated Standards of Reporting Trials.

Table 1
Baseline participant demographics and characteristics (safety analysis set).

Parameter	Riociguat ($n = 14$)	Placebo ($n = 7$)	Total ($n = 21$)
Male, n (%)	10 (71)	6 (86)	16 (76)
Race, n (%)			
White	14 (100)	6 (86)	20 (95)
Asian	0	1 (14)	1 (5)
Black	0	0	0
American Indian or Alaska Native	0	0	0
Native Hawaiian or Other Pacific Islander	0	0	0
Ethnicity, n (%)			
Hispanic or Latino	0	0[a]	0[a]
Age, years	27.1 (6.9)	29.1 (7.2)	27.8 (6.9)
Body mass index, kg/m^2	22.9 (3.4)	21.7 (2.0)	22.5 (3.0)
ppFEV$_1$	67 (12)	64 (13)	66 (12)
Pancreatic insufficiency[b], n (%)	13 (93)	7 (100)	20 (95)

Data are mean (SD) unless otherwise stated.
CF, cystic fibrosis; ppFEV$_1$, percent predicted forced expiratory volume in 1 second.
[a] One participant in the placebo group did not report their ethnicity.
[b] Medical history finding of "pancreatic insufficiency" and "exocrine pancreatic insufficiency" and "CF-related exocrine pancreatic insufficiency."

Due to the unexpected increase in sweat chloride concentration from baseline to Days 14 and 28, particularly in the placebo arm, a sensitivity analysis using a Bayesian approach was performed to evaluate the robustness of the primary analysis using sweat chloride concentration measured 14 days after the last treatment as a surrogate for baseline. This sensitivity analysis also showed no evidence for a treatment-related reduction in sweat chloride concentration. The treatment effect of riociguat versus placebo at Day 28 was estimated to be between –8.7 and 6.4 mmol/L with 90% probability (90% credible interval) and a point estimate (median of posterior distribution) at –1.1 mmol/L (Table 2).

3.2.2. Secondary endpoints

For all secondary endpoints, no clinically relevant or statistically significant changes were observed with riociguat treatment versus placebo (Fig. 2b–d). Change (SD) from baseline for mean ppFEV$_1$ on Day 14 and Day 28 with riociguat ($n = 9$) was +0.9% (4.6) and –0.8% (6.0), respectively; for placebo ($n = 7$), change from baseline was +2.0% (7.3) and +2.4% (9.6), respectively (Fig. 2b). For nasal potential difference, the mean total CFTR-dependent chloride secretion (also termed change with chloride-free isoproterenol) on Day 14 and Day 28 change (SD) from baseline with riociguat

($n = 7$) was +1.6 (7.4) mV and –2.6 (7.3) mV, respectively; for placebo ($n = 5$), change from baseline was +4.4 (12.9) mV and –2.0 (4.2) mV, respectively (Fig. 2c). Data for additional nasal potential difference measures can be found in Supplementary Table 2. Change (SD) from baseline for mean LCI$_{2.5}$ on Day 14 and Day 28 with riociguat was +0.5 (2.1) ($n = 7$) and +0.3 (2.7) ($n = 8$), respectively; for placebo ($n = 6$), change from baseline was –1.0 (2.1) and –0.4 (3.1), respectively (Fig. 2d).

3.3. Riociguat safety profile

All 21 participants were evaluated for safety, of whom 13 (93%) and 7 (100%) in the riociguat and placebo arms, respectively, experienced an AE (Table 3); most AEs were mild to moderate in intensity. No participants died. Serious AEs (SAEs) were reported in one participant each in the riociguat (7%) and placebo (14%) arms. One riociguat-treated participant (7%) experienced distal intestinal obstruction syndrome, an SAE of moderate intensity that was considered drug-related and required hospitalization; the event resolved after three days. One placebo participant (14%) experienced an infective pulmonary exacerbation of CF, an SAE of moderate intensity that was considered unrelated to riociguat. The most frequently

Table 2

Estimated treatment response and treatment effect for change from baseline in sweat chloride concentration with placebo or riociguat treatment (sensitivity analysis using follow-up data as a surrogate for baseline data[a]).

	Treatment response		Treatment effect	
	Change from baseline in sweat chloride concentration, median mmol/L (90% CI)	Probability for an increase/decrease from baseline in sweat chloride concentration, %	Difference between riociguat and placebo in sweat chloride concentration from baseline, median mmol/L (90% CI)	Probability for an increase/decrease from baseline in sweat chloride concentration with riociguat vs placebo, %
Placebo	1.3 (−4.2 to 6.9)	65.1/34.9	–	–
Riociguat 0.5 mg tid for 14 days (Day 14)	3.9 (−1.7 to 9.3)	87.6/12.4	2.6 (−5.0 to 10.0)	71.4/28.6
Riociguat 1.0 mg tid for 14 days (Day 28)	0.2 (−5.3 to 5.7)	52.5/47.5	−1.1 (−8.7 to 6.4)	40.3/59.7

90% CI defined by the 5%- and 95%-quantiles of the posterior probability as lower and upper limits, respectively. CI, credible interval; tid, three times daily.

[a] Sweat chloride concentration measured 14 days after the last treatment was used as a reference for off-treatment measurements and as a surrogate for baseline.

Table 3

Summary of safety and frequently occurring (>10%) drug-related AEs in participants receiving riociguat or placebo, not treated with lumacaftor plus ivacaftor.

AE[a], n (%)	Riociguat (n = 14)	Placebo (n = 7)	Total (n = 21)
Any AE	**13 (93)**	**7 (100)**	**20 (95)**
Study drug-related AEs	8 (57)	4 (57)	12 (57)
AEs leading to study drug discontinuation	2 (14)	0	2 (10)
Maximum intensity for any study drug-related AE			
Mild	1 (7)	3 (43)	4 (19)
Moderate	6 (43)	1 (14)	7 (33)
Severe	1 (7)	0	1 (5)
Any SAE	**1 (7)**	**1 (14)**	**2 (10)**
Study drug-related SAEs	1 (7)	0	1 (5)
SAEs leading to study drug discontinuation	1 (7)	0	1 (5)
AEs of special interest	**1 (7)**	**0**	**1 (5)**
Deaths	**0**	**0**	**0**
Any study drug-related AE	**8 (57)**	**4 (57)**	**12 (57)**
Abdominal pain	3 (21)	0	3 (14)
Cough	3 (21)	1 (14)	4 (19)
Diarrhea	2 (14)	0	2 (10)
Dizziness	1 (7)	1 (14)	2 (10)
Eye allergy	0	1 (14)	1 (5)
Fatigue	2 (14)	1 (14)	3 (14)
Flushing	0	1 (14)	1 (5)
Gastroesophageal reflux disease	2 (14)	0	2 (10)
Headache	3 (21)	2 (29)	5 (24)
Increased bronchial secretion viscosity	0	2 (29)	2 (10)
Increased sputum	1 (7)	1 (14)	2 (10)
Paresthesia	0	1 (14)	1 (5)
Nausea	0	2 (29)	2 (10)

AE, adverse event; SAE, serious AE.

[a] Treatment-emergent.

reported drug-related AEs in the riociguat arm were headache, cough, fatigue, diarrhea, gastroesophageal reflux disease, and abdominal pain (Table 3). Overall, two participants (10%) discontinued riociguat treatment due to an AE: one participant (5%) due to distal intestinal obstruction syndrome (mentioned above) and one participant (5%) due to severe headache. In the riociguat arm, one participant (7%) experienced a single self-terminating event of hemoptysis on Day 28 which was considered drug-related; the event was mild and required no additional treatment.

Due to riociguat's mode of action, symptomatic hypotension was assessed as an AE of special interest. Overall, there was no clinically meaningful difference in mean blood pressure or heart rate between the placebo and riociguat arms. No participants required dose adjustment due to changes in blood pressure. No participants experienced symptomatic hypotension. Systolic blood pressure <95 mmHg was reported in three participants (21%) in the riociguat arm; however, no AEs of hypotension were reported in these participants. Two participants (one in the placebo arm [14%] and one in the riociguat arm [7%]) reported AEs of dizziness, and one participant in the riociguat arm (7%) reported an AE of orthostatic intolerance that was mild in intensity and resolved without additional treatment or change in riociguat dose, and did not reoccur after the dose increase to 1.0 mg tid.

3.4. Decision for early termination

The assessment of unblinded data by the Data Safety Monitoring Board revealed no safety concerns; therefore, based on these data, continuation to Part 2 was approved. However, in spite of an acceptable safety profile, due to the lack of efficacy in Part 1 and the significant changes in the overall landscape of CF therapeutic developments, continuation of the study was deemed unfeasible, and the study was terminated early.

4. Discussion

This study investigated whether riociguat could be an efficacious CFTR modulator and a treatment option for Phe508del homozygous adults with CF. The rationale of this study was based on preclinical studies of PDE5i [11,12] and an open-label Phase I/II pilot study [22,23] of sildenafil which suggested a potential therapeutic role in CF [24–26], and data related to the mode of action of riociguat [7,9], in particular the preclinical data presented in the supplement. These preclinical data demonstrated that sGC stimulators, including riociguat, can modulate the activity and processing of the Phe508del CFTR protein *in vitro* and significantly increase the salivary secretion rate and nasal potential difference, and de-
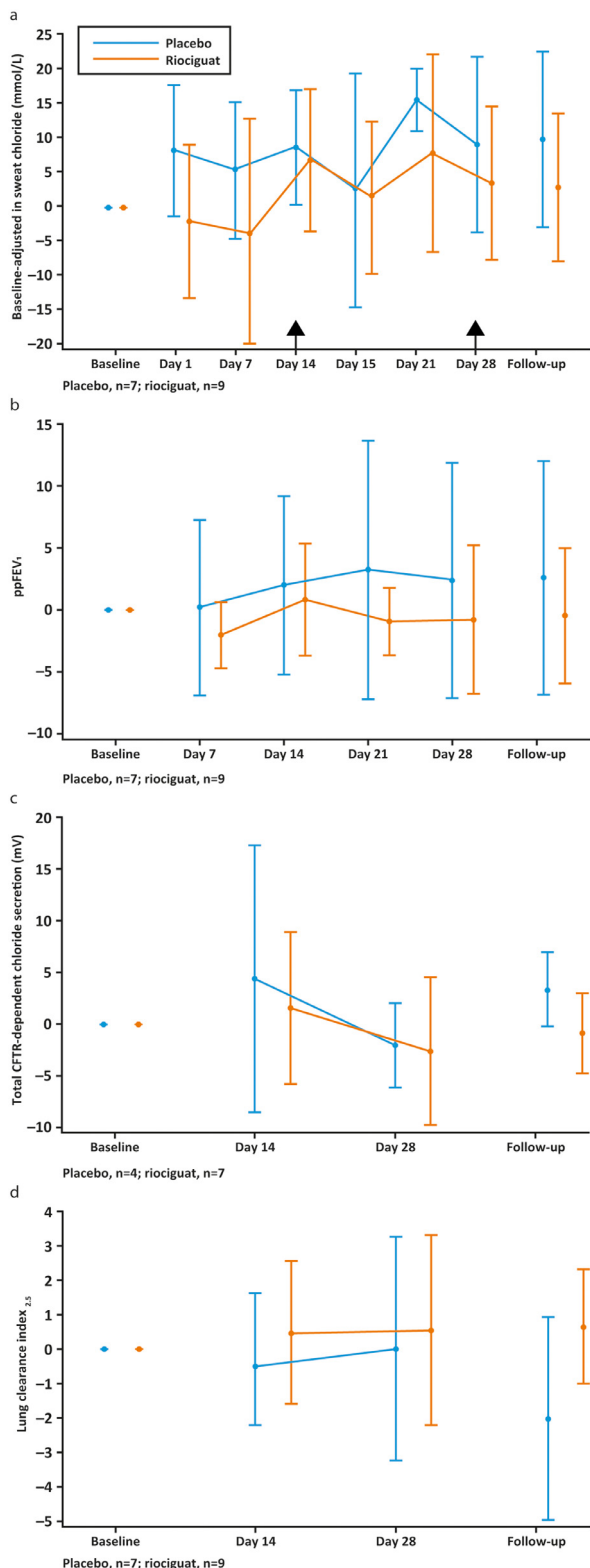
Fig. 2. Baseline-adjusted changes of (a) sweat chloride concentration, (b) ppFEV$_1$, (c) nasal potential difference (total CFTR-dependent chloride secretion, also termed change with chloride-free isoproterenol), and (d) lung clearance index$_{2.5}$ in participants receiving riociguat or placebo, not treated with lumacaftor plus ivacaftor. Data are presented as mean ± SD. Arrows at Day 14 and Day 28 indicate the change in dose of riociguat.

At Day 14 the dose was changed from 0.5 mg to 1.0 mg riociguat.

At Day 28, riociguat was withdrawn.

CFTR, cystic fibrosis transmembrane conductance regulator; ppFEV1, percent predicted forced expiratory volume in 1 s; SD, standard deviation.

crease the salivary chloride content in Phe508del CFTR transgenic mice *in vivo*. However, escalating doses of riociguat up to 1.0 mg tid in Phe508del homozygous adults with CF in Part 1 of Rio-CF were not associated with an improvement in biomarkers of CFTR function, nor in pulmonary function efficacy parameters when compared with placebo. This suggests that preclinical assessments of CFTR restoration of Phe508del in murine models of CF have the propensity to overestimate CFTR activity, perhaps due to less severe processing and trafficking defects [27], as opposed to primary human bronchial epithelial cell models that have been more predictive in this regard.

Systemic riociguat exposure in participants with CF was in the predicted range based on the literature and respective modeling. However, with the limited data available it remains unclear whether the observed exposure was sufficient to achieve detectable efficacy. Whilst caution should be used in interpreting safety data, clinically detectable signs such as changes in blood pressure suggest the dose was sufficient for at least some participants with CF. The potential impact of a higher dose of riociguat on efficacy was not assessed. It is also possible that riociguat may require the addition of a potentiator to demonstrate efficacy, or that *in vitro* and animal models of efficacy for PDE5i may not adequately predict clinical response due to differences in sGC expression among tissues [23]. Further studies would be needed to investigate the translatability of disease models for the sGC pathway, for example, in bronchial epithelial cell lines, or organoid models.

The small increase in sweat chloride concentration in both treatment groups was unexpected based on data from recent studies [28–31]. While sweat chloride concentration is a well-established diagnostic tool for CF, sweat chloride tests do exhibit a known variance [32,33]. The secondary efficacy endpoint, ppFEV$_1$, did not show a positive response with riociguat. Likewise, no improvement was observed in the exploratory endpoints of nasal potential difference and LCI$_{2.5}$. However, as the study was underpowered for analysis of these endpoints, no firm conclusions can be drawn.

Safety analyses showed that riociguat was generally tolerated in adults with CF homozygous for Phe508del, with most AEs being of mild-to-moderate intensity. The safety profile of riociguat in CF was consistent with that previously observed in participants with PAH and CTEPH [34,35], with no new safety signals identified.

At the conclusion of the Rio-CF study, the treatment landscape for CF had changed markedly, with the approval of lumacaftor plus ivacaftor [36]. More recently, a triple combination of elexacaftor, ivacaftor, and tezacaftor has been shown to provide clinical benefit over tezacaftor plus ivacaftor alone [37] and has been approved by the FDA for people with CF ≥6 years with at least one copy of Phe508del [38]. Use of CFTR modulator combinations was evolving as the standard of care in this population [39]; therefore, further clinical development would need to evaluate riociguat in combination with existing CFTR modulators, and thus the planned second part was deemed unfeasible.

Despite the early termination, Rio-CF proved to be a productive global collaboration of experts and research networks, serving as an early model of the use of experienced investigators to aid in the design and implementation of pharmaceutical studies along with global standardization of read-outs which were exploratory at the time of this study. An important limitation of the Rio-CF design was that the sample size assumed a large treatment effect of riociguat. The lower limit of the 90% credible interval for the treatment effect of riociguat versus placebo at Day 28 of −8.7 mmol/L (Table 1) suggests an insufficient effect in this study, compared with reductions observed in other clinical trials [40]. Additionally, the small sample size limited the interpretation of the exploratory endpoints. The secondary endpoints were originally implemented to provide additional guidance in case of ambiguous read-out of

the primary endpoint. However, it became apparent during the study that these endpoints together with the within-group escalation design increased the patient and operational burden in the study, thereby impacting enrollment. As a consequence, these endpoints were declared optional to facilitate completion of the study. Based on this experience, future early clinical studies should more carefully balance the need for signal generation versus the overall burden of a trial in this patient population. Other limitations included the narrow dose range tested, and the short treatment duration. Accordingly, the current data set should be interpreted with caution, as firm conclusions cannot be drawn from the Rio-CF study regarding the safety and efficacy of riociguat therapy in adults with CF homozygous for Phe508del.

Data availability statement

Availability of the data underlying this publication will be determined according to Bayer's commitment to the EFPIA/PhRMA "Principles for responsible clinical trial data sharing." This pertains to the scope, time point, and process of data access. As such, Bayer commits to sharing upon request from qualified scientific and medical researchers' participant-level clinical trial data, study-level clinical trial data, and protocols from clinical trials in participants for medicines and indications approved in the United States (US) and European Union (EU) as necessary for conducting legitimate research. This applies to data on new medicines and indications that have been approved by the EU and US regulatory agencies on or after January 1, 2014.

Interested researchers can use www.clinicalstudydatarequest.com to request access to anonymized participant-level data and supporting documents from clinical studies to conduct further research that can help advance medical science or improve participant care. Information on the Bayer AG criteria for listing studies and other relevant information is provided in the Study sponsors section of the portal. Data access will be granted to anonymized participant-level data, protocols, and clinical study reports after approval by an independent scientific review panel. Bayer AG is not involved in the decisions made by the independent review panel. Bayer AG will take all necessary measures to ensure that participant privacy is safeguarded.

Declaration of Competing Interest

N. Derichs received a speaker honorarium from Vertex Pharmaceuticals, Inc. for participation in a symposium, and served as a consultant for Vertex Pharmaceuticals, Inc. at educational activities and advisory boards. **J.L. Taylor-Cousar** reports grants paid to her institution from the Cystic Fibrosis Foundation; grants paid to her institution and consulting and speaking fees from Vertex Pharmaceuticals, Inc.; grants paid to her institution from Bayer; grants, consulting, and speaking fees from Celtaxys; grants paid to her institution and consulting fees from Proteostasis Therapeutics, Inc.; consulting fees from Santhera, 4DMT, AbbVie, and Polarean; and grants paid to her institution from Eloxx Pharmaceuticals. **J.C. Davies** reports grants from the CF Trust and other funding from Algipharma AS, Bayer AG, Boehringer Ingelheim Pharma GmbH & Co. KG, Galapagos NV, ImevaX GmbH, Nivalis Therapeutics, Inc., ProQR Therapeutics III B.V., Proteostasis Therapeutics, Inc., Raptor Pharmaceuticals, Inc., Vertex Pharmaceuticals, Inc., Enterprise, Novartis, Pulmocide, Flatley, Nivalis Therapeutics, Inc., and Teva. **I. Fajac** received speaker honoraria from Vertex Pharmaceuticals, Inc. for participation in symposia and served as a consultant for Novartis, Proteostasis Therapeutics, Inc., and Vertex Pharmaceuticals, Inc. **E. Tullis** reports grants paid to her institution from Vertex Pharmaceuticals, Inc., Proteostasis Therapeutics, Bayer AG, Boehringer Ingelheim, AbbVie, Celtaxis, Corbus, and Spyryx, and consultancy fees and honoraria from Vertex Pharmaceuticals, Proteostasis Therapeutics, Astra Zeneca and Horizon. **D. Nazareth** has nothing to disclose. **D.G. Downey** reports grants from Vertex Pharmaceuticals, Inc., Proteostasis Therapeutics, Inc., Gilead, and Chiesi; and honoraria or speaker fees from Vertex Pharmaceuticals, Inc., Proteostasis Therapeutics, Inc., and Chiesi. **D. Rosenbluth** has nothing to disclose. **A. Malfroot** has nothing to disclose. **C. Saunders** has nothing to disclose. **R. Jensen** has nothing to disclose. **G.M. Solomon** reports work on advisory boards for Bayer and Electromed. **F. Vermeulen** has nothing to disclose. **A. Kaiser** is an employee of Bayer AG. **S. Willmann** is an employee of Bayer AG. **S. Saleh** is an employee of Bayer AG. **K. Droebner** is an employee of Bayer AG. **P. Sandner** is an employee of Bayer AG. **C.E. Bear** has nothing to disclose. **A. Hoffmann** is an employee of Bayer AG. **F. Ratjen** reports other consultancy fees from Vertex Pharmaceuticals, Inc., Bayer, Talecris, CSL Behring, Roche, and Gilead; grants and consultancy from Novartis; and developed speaker bureau for Genentech outside the submitted work. **S.M. Rowe** reports grants and consulting fees from Arrowhead, Bayer, Celtaxsys, Galapagos/AbbVie, Novartis, Renovion, Synedgen/Synspira, andVertex Pharmaceuticals Inc.; consulting fees from Arcturus, and Cysteic Medicines; and grants from AstraZeneca, Eloxx, N30 Pharmaceuticals, PTC Therapeutics, Translate Bio, and Proteostasis Therapeutics, Inc.

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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.2021.07.015.

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