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Early View

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Efficacy and safety of inhaled ENaC inhibitor BI 1265162 in patients with cystic fibrosis: BALANCE–CF™ 1 – a randomised, Phase II study

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Take home message

Phase I trials showed that single and multiple doses of the inhaled ENaC inhibitor BI 1265162 are safe. In this Phase II trial in patients with CF, BI 1265162 was also safe but did not demonstrate clinically relevant efficacy. The trial was terminated.

Abstract

Background: Inhibition of the epithelial sodium channel (ENaC) in cystic fibrosis (CF) airways provides a mutation-agnostic approach that could improve mucociliary clearance in all CF patients. BI 1265162 is an ENaC inhibitor with demonstrated preclinical efficacy and safety already demonstrated in humans.

Objective: We present results from BALANCE-CF™ 1, a Phase II, placebo-controlled, randomised, double-blind study of four dose levels of BI 1265162 versus placebo for 4 weeks on top of standard of care in adults and adolescents with CF.

Results: Initially, 28 randomised subjects (n=14 each BI 1265162 200 µg BID, placebo BID) were assessed at an interim futility analysis. Compared with placebo, numerical changes of –0.8% (95%CI –6.6, 4.9) in ppFEV$_1$ and +2.1 units (95%CI –2.4, 6.5) in LCI were observed in the active group, meeting a predefined stopping rule; accordingly, the study was terminated. Recruitment had continued during the interim analysis and pending results; 24 patients were added across three dose levels and placebo. The final results including these patients (+1.5% ppFEV$_1$, 200 µg BID dose versus placebo) were not supportive of relevant clinical effect. LCI change was also not supportive, although interpretation was limited due to insufficient traces meeting quality criteria. A 9.4-point improvement in CFQ-R Respiratory Domain was observed in the 200 µg BID dose group versus placebo. BI 1265162 up to 200 µg BID was safe and well-tolerated. Pharmacokinetics were similar to those in healthy volunteers.

Conclusion: BI 1265162 was safe, but did not demonstrate a potential for clinical benefit. Development has been terminated.

Keywords: ENaC inhibitor; Phase II; BALANCE–CF™ 1; efficacy; safety; PK

Introduction

Cystic fibrosis (CF) is a multisystem, life-threatening, autosomal recessive genetic disease resulting from mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene, which encodes the apical cell membrane CFTR anion channel protein [1, 2]. Mutations in *CFTR* result in a defective or absent ion channel that secretes reduced levels of chloride and bicarbonate [1-4]. CFTR dysfunction and/or proteolytic activation by host- and bacteria-derived proteases in CF lead to hyperactivation of the epithelial sodium channel (ENaC) [5-11]. This, in turn, leads to reduced airway surface liquid volume, dehydrated mucus and dysfunctional cilia, resulting in poor mucociliary clearance (MCC) [1, 12]. Poor MCC leads to mucus obstruction, chronic airway inflammation, and infection with bacterial pathogens [13].

CFTR modulators address the underlying ion transport defect in CF [14]. Currently, approved CFTR modulators include the potentiator ivacaftor (for patients with at least one *G551D* allele, other CFTR gating mutations and responsive mutations based on clinical and/or *in vitro* assay data); the corrector/potentiator combinations lumacaftor/ivacaftor (for patients homozygous for the *F508del* mutation) and tezacaftor/ivacaftor (for patients homozygous for the *F508del* allele, those with an *F508del* allele plus residual-function mutation and responsive mutations based on clinical and/or *in vitro* assay data); and the triple-agent CFTR modulator elexacaftor/tezacaftor/ivacaftor (for patients with at least one *F508del* allele and responsive mutations based on *in vitro* assay data). In clinical studies, CFTR modulators have improved percentage predicted forced expiratory volume in 1 second (ppFEV$_1$) by 3–14% [15-22], with a sustained effect confirmed in open-label extension studies [23, 24]. A real-world study has demonstrated a slowed decline of ppFEV$_1$ over 5 years [25].

However, for most patients with CF, an improvement in pulmonary function is not necessarily a return to normal and exacerbations still occur, albeit at a lower rate [15, 24, 25]. In addition, bacteria are not eradicated from the airways over time [25-27]. Treatments that target ENaC in addition to CFTR modulators could assist in further normalising airway surface hydration [28] by providing an enhanced electrical driving force favouring CFTR-mediated chloride secretion, restoring ion and water homeostasis [1, 29]. Furthermore, CFTR modulator therapy is not approved for approximately 5–10% of patients with CF because their mutations lead to an unresponsive CFTR protein [30]. In countries such as Brazil, Israel, Italy and Turkey, over 30% of patients with CF do not possess an *F508del* allele [31, 32]; ENaC inhibition in these regions represents an even more significant therapeutic option. Therefore, ENaC inhibition is an important, mutation-agnostic therapeutic approach that could operate independently of CFTR function and mutation class [1, 30].

BI 1265162 is an ENaC inhibitor inhaled via the Respimat® Soft Mist™ inhaler (SMI). BI 1265162 has demonstrated preclinical efficacy [33] and safety in healthy volunteers [34]. The objectives of this study were to assess the efficacy, safety and pharmacokinetics of 20 µg, 50 µg, 100 µg and 200 µg twice-daily (BID) doses of BI 1265162 (BI 20, BI 50, BI 100, and BI 200) via the Respimat® SMI, compared with placebo BID (PBO), as an add-on to standard CF therapies in patients aged ≥12 years old.

Methods

A summary of methods is provided. A full description can be found in the online supplement, available at [insert link once available].

This was a multicentre, multinational, randomised, double-blind, placebo-controlled, parallel-group, dose-ranging study (Figure 1). Ninety-eight patients from 12 years of age were planned for randomisation. The start of adolescent patients' enrolment was to be based on review of adult safety data, carried out by an independent data monitoring committee (DMC) in collaboration with the CF Foundation.

The primary endpoint was the change from baseline after 4 weeks of treatment in trough (30 minutes pre-dosing) $ppFEV_1$. Secondary endpoints were change from baseline after 4 weeks of treatment in: i) lung clearance index (LCI), ii) Cystic Fibrosis Questionnaire – Revised (CFQ-R) [35] total score and iii) Cough and Sputum Assessment Questionnaire (CASA-Q©) [36], adverse events (AEs) and pharmacokinetics.

An interim futility analysis on the first 28 patients (BI 200 or PBO) was planned to assess potential for efficacy and to prevent exposure of further patients in case of insufficient potential. Per protocol, recruitment continued pending results of the interim analysis to enable the study to be carried out in the most time-efficient manner. A decision on termination was to be made if the increase in trough $ppFEV_1$ % was <1.5% and the decrease (improvement) in LCI was <0.3 units (futility).

The planned analyses for proof of concept and dose-finding were to use multiple comparison and modelling techniques to measure the difference between the PBO and active treatment. Power calculations for the final analysis were to be based on

having $ppFEV_1$ results for at least 24 evaluable patients each for the BI 200 and PBO groups and at least 12 evaluable patients each for all other groups.

A restricted maximum likelihood-based approach using a mixed model with repeated measurements (MMRM) was carried out to assess the change from baseline in trough $ppFEV_1$. Visits were treated as the repeated measure with an unstructured covariance structure used to model the within-patient measurements. Analysis of covariance (ANCOVA) with adjustment for categorical effects of treatment and the fixed continuous effect of baseline was carried out to assess change from baseline in LCI. Patient-reported outcomes were descriptive in nature.

Pre-specified sensitivity analyses to address any outlier data points and expected variability were carried out for both $ppFEV_1$ and LCI endpoints. Data were reviewed by an Interim Analysis Assessment Committee (Boehringer Ingelheim internal, independent from the study team) at the interim futility analysis for the impact of outliers.

A model-based predefined subgroup analysis was performed to investigate any impact of patient characteristics, CFTR mutation status and concomitant CF therapy use on the change from baseline in trough $ppFEV_1$.

Results

Study population

Patient disposition is described in Figure 2. Baseline characteristics and medication use were balanced between groups and are summarised in Table 1.

Due to the COVID-19 pandemic there was a temporary halt in recruitment just prior to the interim futility results. This further added to the limitation in sample size beyond the interim analysis. A total of 52 patients were randomised into the PBO and BI 20, 50, 100 and 200 dosing groups (N=18, 6, 5, 5, 18, respectively) until termination. Forty-nine patients (94.2%) completed the planned treatment and observation periods. Three (5.8%, two receiving BI 20 and one receiving BI 200) prematurely discontinued study medication and did not complete the planned observation period, due to the COVID-19 pandemic (BI 20) and AEs (BI 200). Embryo-foetal development data were not available at study start, so that Women of Childbearing Potential (WoCBP) were excluded in the initial protocol leading to a male predominant population; embryo-foetal development data allowed inclusion of WoCBP using adequate contraception in a revision of the protocol (see Supplementary Materials). Treatment compliance was high, with mean percentages (standard deviation [SD]) of prescribed medication taken during the treatment period ranging from 93.2 (17.7) in the BI 20 group to 100.4 (4.8) in the BI 100 group, with no relevant difference between groups.

All enrolled patients were adults. Enrolment of adolescents was approved by the independent DMC but was not possible because of recruitment stop due to the COVID-19 pandemic and the interim futility analysis.

Efficacy

All efficacy data presented below are after 4 weeks' treatment.

Interim analysis

Results from the interim analysis of BI 200 versus PBO for $ppFEV_1$ and LCI (n=14 vs n=14, and n=3 vs n=6, respectively) are presented in Table 2.

An adjusted mean (standard error [SE]) decrease in trough $ppFEV_1$ of 0.1% (1.95%) was observed in the BI 200 group compared with a 0.7% (2.00%) increase in the PBO group, equating to a numerical difference of –0.8% (95% confidence interval [CI] –6.6 to 4.9).

An adjusted mean (SE) increase in LCI of 0.8 (1.46) units was observed in the BI 200 group compared with a decrease of 1.3 (1.01) units in the PBO group, equating to a numerical difference of 2.1 (95% CI –2.4 to 6.5) units.

Stopping rules defined for the futility analysis were met for this study, recruitment was stopped and the study terminated when these data were available, also concurrent to when recruitment had already been placed on hold due to the COVID-19 pandemic. Thus, hypothesis testing was not carried out and sample size was not adequate to assess dose−response. Statistical analysis of $ppFEV_1$ and LCI is exploratory and descriptive only, and inferences should be made with caution.

Final analysis

Results from the final analysis of treatment with BI 200 (n=16) versus PBO (n=18) for $ppFEV_1$, including sensitivity analyses, are presented in Table 3. At study baseline, mean (SE) $ppFEV_1$ was 59.21% (2.09%). An adjusted mean (SD) increase in trough

ppFEV$_1$ of 0.5% (1.77%) was observed in the BI 200 group compared with –1.0% (1.70%) in the PBO group, equating to a numerical difference of 1.5% (95% CI –3.5 to 6.5).

Descriptive and exploratory statistics for change in trough ppFEV$_1$ for all groups are shown in Table 4 and Supplementary Figure 1a. A numerical mean increase from baseline in trough ppFEV$_1$ was observed in the BI 100 and 200 groups. Trough ppFEV$_1$ was relatively unstable in the PBO and BI 200 groups over the 4-week period (variability extremes of +17.4% and –15.2%; +11.4% and –12.7% in lung function changes, respectively). Individual patient changes from baseline in ppFEV$_1$ are shown in Supplementary Figure 2.

In a sensitivity analysis, five and four patients from the BI 200 and PBO groups, respectively, had ppFEV$_1$ visit data censured due to AEs that could have affected lung function, unacceptable pulmonary function test quality or poor treatment compliance (Supplementary Table 1). The decision to censure the data was made without knowing treatment allocation. Sensitivity analyses did not change the outcome of either the interim or final analyses (a numerical difference in ppFEV$_1$ between BI 200 and PBO groups of 2.7% [95% CI –2.3 to 7.7] in the MMRM analysis and 5.7% [95% CI –1.6 to 12.9] in the quantile regression analysis). Individual patient changes from baseline in ppFEV$_1$ in the sensitivity analyses are shown in Supplementary Figure 3.

Subgroup analysis showed a consistent response pattern of trough ppFEV$_1$ after treatment with BI 1265162 across all subgroups, but no responsive subpopulations were identified (Supplementary Figure 4). A total of 19/52 (36.5%) patients were receiving CFTR modulator therapy at randomisation (7 [38.9%], 3 (50.0%], 2 [40.0%]

and 7 [38.9%] patients in the placebo, BI 20, BI 50 and BI 200 groups, respectively). In the subgroup analysis, patients on BI 200 receiving CFTR modulators demonstrated a mean numerical –1.2% (95% CI -8.8, 6.4) change in $ppFEV_1$ compared with placebo, whereas patients not receiving CFTR modulators in this group demonstrated a numerical 3.1% (95% CI –4.2, 10.3) change in $ppFEV_1$ compared with placebo (Supplementary Figure 4). The confidence intervals of the subgroups were overlapping.

At study baseline, 16 patients performed valid LCI tests, with a mean (SE) score of 14.68 (1.06) units. At Week 4, only 11 patients performed valid LCI tests; treatment with BI 200 (n=3) resulted in an adjusted mean increase (SE) in LCI of 0.8 (1.46) units, compared with a decrease of 1.3 (1.01) units in the PBO group (n=6; ANCOVA analysis), equating to a numerical difference of 2.1 units (95% CI –2.4 to 6.5).

Descriptive and exploratory statistics for change in LCI for all groups are shown in Table 4 and Supplementary Figure 1b. Supplementary Figure 5 describes individual patient changes from baseline in LCI. The LCI analysis is limited given the small number of LCI values that could be obtained across the study.

Patient-reported outcomes

The mean CFQ-R total score increased (improved) for all groups except BI 100 (Supplementary Table 2). For CFQ-R Respiratory Domain, the BI 20, 100 and 200 groups met the minimal clinically important difference (+4 points) outcomes for patients with stable CF [37] (mean [SD] scores 6.94 [5.32], 6.67 [13.26] and 6.60 [14.93], respectively).

There was no correlation between change in CFQ-R Respiratory Domain score and change in $ppFEV_1$ (Supplementary Figure 6), but sample sizes were limited.

The mean Cough and Sputum Symptom Domain score of the CASA-Q® increased, showing numerical improvement for patients across all groups; however, no consistent dose-dependent trends were observed with no apparent dose dependence (Supplementary Table 3).

Safety

Overall AEs are summarised in Table 5. Drug-related AEs were reported for 16.7%, 0%, 20.0%, 20.0% and 27.8% of patients in the PBO, and BI 20, 50, 100 and 200 groups, respectively. There was a low incidence of CF exacerbations (1/18 patients each [5.6%] in both the placebo and BI 200 groups).

AEs for >1 patient in any treatment group are detailed in Table 6. An AE of special interest (AESI), hyperkalaemia, was reported for two patients (PBO, n=1; BI 200, n=1). This was not considered serious and did not lead to dose reduction or discontinuation. One patient in the BI 200 group discontinued due to chest discomfort of mild intensity on Study Days 2–4. This was considered to be drug related by the investigator. However, this event was not considered a serious AE (SAE) or an AESI. Two patients had SAEs (BI 200, n=1 [lung congestion]; PBO, n=1 [hypoglycaemia with a fatal outcome after the end of the treatment period]).

Pharmacokinetics

Results of pharmacokinetics analyses are shown in Supplementary Table 4. Steady-state mean concentration profiles at Day 8 (Visit 3) showed fast absorption across all groups. Mean maximal concentration (C_{max}) and area under the concentration–time curve from 0 to 4 hours (AUC_{0-4}) at Visit 3 increased almost proportionally for the BI 20, 50 and 100 groups. The mean trough concentrations of BI 1265162, as well as drug concentrations at 5 minutes after inhalation ($C_{0.083}$), were similar across individual patients and groups, with some exceptions. The variability for C_{max} and AUC_{0-4} was high for the BI 200 group (81.5% and 71.0% geometric coefficient of variance, respectively), and lower for the other groups (ranging from 20.1% and 8.93%, respectively in the BI 100 group to 57.0% and 45.3%, respectively in the BI 20 group).

Discussion

The aim of the study was to investigate the efficacy, safety and pharmacokinetics of the ENaC inhibitor BI 1265162 in adult and adolescent patients with CF versus placebo.

The independent DMC proposed to enrol adolescents, but due to a COVID-19 pandemic-driven stop of enrolment and then termination of the study based on results of a futility analysis, adolescent patients were not enrolled. In addition, due to the early stopping of the study, sample sizes, especially in the lower-dose groups, were small, and no hypothesis testing of dose–response could be carried out.

Due to an insufficient effect on trough $ppFEV_1$ and LCI after 4 weeks of treatment at an interim futility analysis, and also limited potential for effect in the sensitivity analyses, the study was terminated. There was also no significant effect in the larger data set of completed patients (including those enrolled during the analysis of interim data). No response characteristics could be identified. Subgroup analysis in this study did not suggest an impact of concomitant, stable CFTR modulator therapy on $ppFEV_1$ changes seen with treatment with BI 1265162. Small sample sizes of the subgroups, however, do not allow any stringent conclusion. No dose-dependent trends in improvements in patient-reported outcomes were observed, although clinically relevant changes compared with PBO were observed for the BI 20, 100 and 200 groups. There was no correlation between change in $ppFEV_1$ and change in CFQ-R Respiratory Domain scores at 4 weeks, although the sample size was relatively small. Improvement in patient-reported outcomes is not always correlated with improvements in lung function. In a Phase Ib study of the antisense oligonucleotide eluforsen in patients with *F508del*/*F508del* CF, at least minimal clinically important difference (+4 points) in CFQ-R Respiratory Symptom Score was achieved in two dose groups of a multiple-ascending-dose cohort compared with placebo, but this was not related to any meaningful change in $ppFEV_1$ [38]. In an analysis of lung function changes and signs and symptoms of pulmonary exacerbations in patients with CF in the STOP study, only an extremely weak correlation between $ppFEV_1$ and Chronic Respiratory Infection Symptom Score (R^2=0.157; $p<0.001$) was observed [39].

Occurrence of drug-related AEs was similar, and occurrence of CF exacerbations was low, across treatment groups. No clinically relevant changes from baseline in vital signs and physical examinations were observed. Occurrence of drug-related

AEs was low and comparable across PBO and BI 1265162 groups. As might be expected for patients with CF, the most frequently reported system organ classes were respiratory, thoracic and mediastinal disorders, and infections and infestations, which are commonly reported in studies of CF therapies and may be related to underlying disease. Two cases of hyperkalaemia were reported (PBO, n=1; BI 200, n=1). This AE deserves special attention as it could be caused by renal activity of BI 1265162 due to high levels of ENaC expression in the kidney [1], and previous clinical development of ENaC inhibitors has been hampered by hyperkalaemia [29]. One patient in the Phase I study of BI 1265162 had hyperkalaemia [34]; however, renal blockade of ENaC was considered unlikely given the urinary electrolyte values in that subject. The cases of hyperkalaemia reported in this study were not considered serious, and did not lead to dose reduction or discontinuation. The overall AE evaluation did not indicate a higher risk for respiratory or infectious AEs in the active treatment arms.

On one hand, the ppFEV$_1$ and LCI cut-off values at the interim analysis were based on statistical calculations of having a high probability for the study succeeding and achieving a clinically meaningful improvement with N=14 each in PBO and BI 200 groups based on the assumed treatment effect. On the other hand, the cut-off was chosen to have good chances to stop the trial early assuming no treatment effect. Based on the ppFEV$_1$ signal observed at the interim, reaching a substantial lung function improvement was not expected to occur in this study with continued recruitment. The probability of achieving the original goal was re-evaluated conditioned on the observed results and number of patients (original analysis and including the additional patients) and confirmed a low probability of success even with the original assumptions for the treatment effect. A 9% predicted probability of

reaching the targeted 4% improvement in ppFEV$_1$ was calculated based on the available 52 randomized patients if the study would have continued and fully recruited.

Previous failures of inhaled ENaC inhibitors in clinical studies may have been due to inadequate dosing and/or bronchiolar deposition in patients with heterogeneous airway plugging. The dose used in the current clinical study was based on fluid absorption data from a rat model (BI 1265162 was tracheally instilled) and MCC data from a sheep model (BI 1265162 was nebulised) [40], also correcting for lung deposition using the Respimat® SMI in humans [41]. Nevertheless, underdosing in this study cannot be ruled out, without a more direct measure of ENaC function in the airways and because animal studies were carried out in models that had no mucus plugging or structural lung damage as seen in patients with CF. Therefore, the dose and duration of inhaled ENaC inhibitor required for a therapeutic benefit may have been underestimated.

This study had a number of adaptive steps that allowed early termination, with a number of design elements that could be considered or reconsidered for other studies.

Recruitment was continued during analysis of interim data. There must be a balance between expediting study completion with a potentially medically valuable drug and continued enrolment into a study of a non-efficacious drug. If efficacy had been greater, several months would have been saved in the programme; however, recruitment of almost half the study population into a study of a likely non-efficacious treatment regimen was avoided.

The decision to terminate was based on statistical considerations, which must be robust enough to handle individual variability, especially in small sample sizes. In our study, the standard deviations for $ppFEV_1$ were as expected, and although a change from a delta of –0.8 to 1.5 % $ppFEV_1$ was observed in the final analysis, the decision to stop the study after the futility analysis was considered correct given the very low probability of reaching the target $ppFEV_1$ with the given study design (duration, dose, potential for efficacy).

Although, as stated above, overall variability was as expected, lung function in the placebo and BI 200 groups was unstable during the study, as indicated by the largest extremes in $ppFEV_1$ values at Week 4 of any treatment group. To increase lung function stability in future studies with potential for better treatment discrimination, an inclusion criterion of variability of $ppFEV_1$ between screening and baseline of <15% could be considered. A longer stability period during run-in, for use of concomitant CF drugs could also be considered.

A longer treatment period would leverage the usage of the MMRM approach and reduce the impact of missing data points, and also account for effects of temporary worsening that can occur in such a fluid disease.

The analysis of change from baseline in LCI contained data from only 20% of patients. This was due to eligibility criteria for this measurement (FEV_1 >60% predicted) and quality control (QC) requirements, which had been set and monitored in close collaboration with Central Over Reading Centres (CORCs) to achieve the highest LCI quality. Of 28 patients who qualified for the N_2 multiple breath washout test at baseline, only 11 patients passed the QC test for LCI at both baseline and Week 4 from a study population of 52. A number of measures could be implemented

to further optimise LCI. Firstly, testing at screening (and not just baseline) would have provided: (i) training opportunity for participants new to the technique; (ii) rapid review of trace quality by the CORC to allow feedback to sites requiring technical improvements ahead of baseline visit; (iii) where LCI is a key outcome and protocol-defined, potential to use screening values in cases where the baseline visit test fails QC. Secondly, in this study, LCI was performed at two visits – baseline and Week 4. Having more than one 'on-treatment' value would minimise any effect of missing data. Thirdly, operational challenges were experienced at some sites with less experience in carrying out the LCI test. When sample sizes are limited based on subgroup eligibility criteria, selecting the most highly skilled sites to perform this measurement would improve the proportion of successful attempts. Highly skilled sites are those that have consistently high success rates, know how to create a suitable testing environment, and observe any abnormalities and act on them accordingly. Finally, data from CFTR modulator studies have shown LCI has superior sensitivity over FEV_1 in early structural lung abnormalities associated with CF, particularly in younger patients.[42-45] In future studies, the utility of LCI will be better in mild-to-moderate versus more severe disease. Conversely, reducing the $ppFEV_1$ threshold for performing LCI to <60% would increase the numbers of eligible patients but increase non-acceptable LCI values with potential for patient and site frustration with the procedure.

Conclusions

Numerous attempts to demonstrate benefit with ENaC inhibition have failed [29], although a recent study with the ENaC antisense oligonucleotide ION-827359 in patients with CF has demonstrated a numerical dose-dependent increase in ppFEV$_1$ after 4 weeks' treatment, with a numerical 4.5% increase in the highest dose group versus placebo [46]. However, on balance, the potential of ENaC inhibition in patients with CF must be questioned. There is a clear medical need for further breakthroughs in CF targeting those patients not eligible for CFTR modulators and for further normalisation of the status of patients who already receive CFTR modulators, with a drive toward simplification of treatment in this polytherapy disease. Whether this is through improvements in modulator approaches, channel approaches, treatment of inflammation, cure via gene therapy approaches, or other modalities, there continues to be a strong need for improvement in therapy.

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Conflicts of interest

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Tables

Table 1. Patient baseline demographics and concomitant drug use – TS

	BID				
	Placebo	BI 20 µg	BI 50 µg	BI 100 µg	BI 200 µg
Number of patients (%)	18 (100.0)	6 (100.0)	5 (100.0)	5 (100.0)	18 (100.0)
Gender, N, (%)					
Male	16 (88.9)	5 (83.3)	2 (40.0)	4 (80.0)	15 (83.3)
Female	2 (11.1)	1 (16.7)	3 (60.0)	1 (20.0)	3 (16.7)
Race, N, (%)					
Asian	1 (5.6)	0	0	0	0
Black or African American	0	0	0	1 (20.0)	0
White	17 (94.4)	6 (100.0)	5 (100.0)	4 (80.0)	18 (100.0)
Region, N, (%)					
North America	3 (16.7)	2 (33.3)	0	2 (40.0)	4 (22.2)
Europe	15 (83.3)	4 (66.7)	5 (100.0)	3 (60.0)	14 (77.8)
Age, years					
Mean (SD)	29.3 (10.1)	26.8 (5.8)	31.2 (8.6)	36.8 (4.2)	33.4 (10.2)
Range (Min to Max)	18 to 48	21 to 34	25 to 42	32 to 42	22 to 50
Height, cm					
Mean (SD)	175.6 (10.4)	173.8 (7.9)	165.0 (11.2)	171.2 (12.4)	171.9 (9.5)
Range (Min to Max)	148 to 197	165 to 184	153 to 177	155 to 187	154 to 189
Weight, kg					
Mean (SD)	68.72 (12.17)	73.35 (12.18)	60.92 (9.71)	66.76 (9.93)	65.95 (9.06)
Range (Min to Max)	50.8 to 90.0	53.0 to 90.0	50.1 to 74.0	55.3 to 80.1	46.0 to 87.8
BMI, kg/m^2					
Mean (SD)	22.19 (2.55)	24.15 (2.71)	22.42 (3.20)	22.74 (1.94)	22.37 (3.08)
Range (Min to Max)	17.0 to 26.9	19.5 to 26.8	18.3 to 26.3	20.8 to 25.4	17.2 to 30.0
CFTR modulator					
No	11 (61.1)	3 (50.0)	3 (60.0)	5 (100.0)	11 (61.1)
Yes	7 (38.9)	3 (50.0)	2 (40.0)	0	7 (38.9)
Highly effective	1 (5.6)	2 (33.3)	0	0	2 (11.1)
Ivacaftor	1 (5.6)	0	0	0	0
Elexacaftor/Ivacaftor/ Tezacaftor	0	2 (33.3)	0	0	2 (11.1)
Not highly effective	6 (33.3)	1 (16.7)	2 (40.0)	0	5 (27.8)
Ivacaftor/Tezacaftor	3 (16.7)	0	2 (40.0)	0	2 (11.1)
Ivacaftor/Lumacaftor	3 (16.7)	1 (16.7)	0	0	3 (16.7)
Hypertonic saline solution					
No	5 (27.8)	1 (16.7)	1 (20.0)	2 (40.0)	4 (22.2)
Yes	13 (72.2)	5 (83.3)	4 (80.0)	3 (60.0)	14 (77.8)
Dornase alfa					
No	5 (27.8)	2 (33.3)	2 (40.0)	3 (60.0)	7 (38.9)
Yes	13 (72.2)	4 (66.7)	3 (60.0)	2 (40.0)	11 (61.1)
Mannitol					
No	18 (100.0)	6 (100.0)	4 (80.0)	3 (60.0)	18 (100.0)
Yes	0	0	1 (20.0)	2 (40.0)	0
Inhaled mucolytic therapy					
No	5 (27.8)	1 (16.7)	1 (20.0)	1 (20.0)	4 (22.2)
Yes	13 (72.2)	5 (83.3)	4 (80.0)	4 (80.0)	14 (77.8)
Inhaled antibiotics					
No	11 (61.1)	2 (33.3)	2 (40.0)	2 (40.0)	9 (50.0)
Yes	7 (38.9)	4 (66.7)	3 (60.0)	3 (60.0)	9 (50.0)
Inhaled bronchodilators					
No	2 (11.1)	0	0	0	2 (11.1)
Yes	16 (88.9)	6 (100.0)	5 (100.0)	5 (100.0)	16 (88.9)

Inhaled corticosteroids					
No	5 (27.8)	3 (50.0)	0	2 (40.0)	6 (33.3)
Yes	13 (72.2)	3 (50.0)	5 (100.0)	3 (60.0)	12 (66.7)

BI: BI 1265162; BID: twice daily; BMI: body mass index; CFTR: cystic fibrosis transmembrane conductance regulator; Min: minimum; Max: maximum; SD: standard deviation; TS: treated set.

Table 2. Change in trough ppFEV$_1$ after 4 weeks of treatment with BI 1265162 200 µg BID – interim analysis

Change from baseline in trough ppFEV$_1$ (MMRM) – TS					
	N	Adjusted mean (%)[1]	SE	95% CI[2]	p-value[2]
Placebo	14	0.7	2.00	–3.4, 4.9	-
BI 200 µg	14	–0.1	1.95	–4.1, 3.9	-
BI 200 µg vs placebo	-	–0.8	2.79	–6.6, 4.9	0.7639
Change from baseline in LCI (ANCOVA) – N$_2$MBWS					
	N	Adjusted mean (%)[1]	SE	95% CI[2]	p-value[2]
Placebo	6	–1.3	1.01	–3.7, 1.2	-
BI 200 µg	3	0.8	1.46	–2.8, 4.4	-
BI 200 µg vs placebo	-	2.1	1.83	–2.4, 6.5	0.3039

[1]Based on MMRM with fixed effects for baseline, visit, treatment, treatment-by-visit interaction, baseline-by-visit interaction, and random effect for patient.

[2]Confidence intervals and p-values are provided for reference only and inference should not be drawn.

ANCOVA: analysis of covariance; BI: BI 1265162; BID: twice daily; CI: confidence interval; LCI: lung clearance index; MMRM: mixed model for repeated measures; N$_2$MBWS: N$_2$ multiple breath washout set; ppFEV$_1$: percent predicted forced expiratory volume in 1 second; SE: standard error, TS: treated set.

Table 3. Change in trough ppFEV$_1$ and LCI after 4 weeks of treatment with BI 1265162 200 µg BID – final analysis

`Change from baseline in trough ppFEV$_1$ (MMRM) – TS					
	N	Adjusted mean (%)[1]	SE	95% CI[2]	p-value[2]
Placebo	18	–1.0	1.70	–4.5, 2.4	-
BI 200 µg	16	0.5	1.77	–3.2, 4.1	-
BI 200 µg vs placebo	-	1.5	2.45	–3.5, 6.5	0.5468
Change from baseline in LCI (ANCOVA) – N$_2$MBWS					
	N	Adjusted mean (units)[3]	SE	95% CI[2]	p-value[2]
Placebo	6	–1.3	1.01	–3.7, 1.2	-
BI 200 µg	3	0.8	1.46	–2.8, 4.4	-
BI 200 µg vs placebo	-	2.1	1.83	–2.4, 6.5	0.3039
Sensitivity analyses					
Change from baseline in trough ppFEV$_1$ – TS					
MMRM, pre-specified					
	N	Adjusted mean (%)[4,5]	SE	95% CI[2]	p-value[2]
Placebo	17	–0.4	1.65	–3.8, 3.0	-
BI 200 µg	15	2.3	1.78	–1.4, 6.0	-
BI 200 µg vs placebo	-	2.7	2.43	–2.3, 7.7	0.2761
Quantile regression, *post hoc*					
	N	Median estimate (%)	SE	95% CI	p-value[2]
All visits[6]					
Placebo	17	54.6	2.2	50.2, 59.0	-
BI 200 µg	16	58.6	1.8	54.8, 62.3	-
BI 200 µg vs placebo	-	4.0	2.8	–1.8, 9.7	0.1728
Visit data excluded[5,6]					
Placebo	14	53.8	2.5	48.7, 58.9	-
BI 200 µg	12	59.4	2.0	55.3, 63.5	-

BI 200 µg vs placebo	-	5.7	3.5	–1.6, 12.9	0.1193

[1]Based on MMRM with fixed effects for baseline, visit, treatment, treatment-by-visit interaction, baseline-by-visit interaction, and random effect for patient.
[2]Confidence intervals and p-values are provided for reference only and inference should not be drawn.
[3]Based on ANCOVA with fixed effects for baseline and treatment.
[4]Based on MMRM with fixed effects for baseline, visit, treatment, treatment-by-visit interaction, baseline-by-visit interaction and random effect for patient.
[5]Data from visits were excluded based on AEs that might have affected pulmonary function tests, compliance, and unacceptable pulmonary function test quality at baseline and/or baseline condition considered as important protocol deviation.
[6]Estimate of median is using overall median of baseline.
AE: adverse event; ANCOVA: analysis of covariance; BI: BI 1265162; BID: twice daily; CI: confidence interval; LCI: lung clearance index; MMRM: mixed model for repeated measures; N_2MBWS: N_2 multiple breath washout set; ppFEV_1: percent predicted forced expiratory volume in 1 second; SE: standard error, TS: treated set.

Table 4. Change in trough ppFEV$_1$ and LCI after 4 weeks of treatment with BI 1265162 200 µg BID – all treatment groups (descriptive statistics)

Trough ppFEV$_1$ – TS								LCI – N$_2$MBWS						
	Baseline score				Change from baseline after 4 weeks			Baseline score				Change from baseline after 4 weeks		
	N	Mean	SD		N	Mean	SD	N	Mean	SD		N	Mean	SD
Placebo	18	59.40	11.29		17	−0.60	8.03	6	13.899	3.581		6	−0.824	3.312
BI 20 µg	6	69.93	15.99		4	−0.50	2.82	0	-	-		0	-	-
BI 50 µg	5	63.02	14.40		5	−0.22	2.62	1	14.958	na[1]		1	−0.238	na[1]
BI 100 µg	5	65.50	7.00		5	2.82	3.57	1	16.223	na[1]		1	−2.547	na[1]
BI 200 µg	17	57.94	13.76		16	0.45	5.42	3	16.254	1.794		3	−0.081	1.001

[1]Data from only one patient; no standard deviation could be calculated.

BI: BI 1265162; BID: twice daily; LCI: lung clearance index; N$_2$MBWS: N$_2$ multiple breath washout set; na: not applicable; ppFEV$_1$: percent predicted forced expiratory volume in 1 second; SD: standard deviation; TS: treated set.

Table 5. Overall summary of patients with adverse events – TS

	BID				
	Placebo	BI 20 µg	BI 50 µg	BI 100 µg	BI 200 µg
	N (%)	N (%)	N (%)	N (%)	N (%)
Total number of patients	18 (100.0)	6 (100.0)	5 (100.0)	5 (100.0)	18 (100.0)
Patients with at least one AE	12 (66.7)	0	2 (40.0)	2 (40.0)	15 (83.3)
Patients with severe AEs	1 (5.6)	0	0	0	0
Patients with drug-related AEs[1]	3 (16.7)	0	1 (20.0)	1 (20.0)	5 (27.8)
Patients with AEs leading to discon.[2]	0	0	0	0	1 (5.6)
Patients with other significant AEs[3]	0	0	0	0	1 (5.6)
Patients with AESIs	1 (5.6)	0	0	0	1 (5.6)
Patients with SAEs	1 (5.6)[4]	0	0	0	1 (5.6)[5]

[1]As defined by the investigator. [2]Discontinuation of study drug. [3]According to ICH E3.
[4]Event (PT hypoglycaemia) was considered serious because it required or prolonged hospitalisation and resulted in death.
[5]Event (PT pulmonary congestion) was considered serious because it was an "other medically important event".
A patient could have had SAEs with multiple seriousness criteria. Percentages were calculated using total number of patients per treatment group as the denominator.
AE: adverse event; AESI: adverse event of special interest; BI: BI 1265162; BID: twice daily; discon.: discontinuation; ICH: International Council for Harmonisation; PT: preferred term; SAE: serious adverse event; TS: treated set.

Table 6. Adverse events (preferred terms) reported for >1 patient in any treatment group – TS

	BID				
	Placebo	BI 20 µg	BI 50 µg	BI 100 µg	BI 200 µg
	N (%)	N (%)	N (%)	N (%)	N (%)
Total number of patients	18 (100.0)	6 (100.0)	5 (100.0)	5 (100.0)	18 (100.0)
Patients with at least one AE	12 (66.7)	0	2 (40.0)	2 (40.0)	15 (83.3)
Gastrointestinal dis.					
Diarrhoea	0	0	1 (20.0)	0	1 (5.6)
Nausea	0	0	0	0	2 (11.1)
General dis. and admin. site conditions					
Chest discomfort	0	0	0	0	2 (11.1)
Fatigue	1 (5.6)	0	0	0	1 (5.6)
Infections and infestations					
Nasopharyngitis	4 (22.2)	0	0	0	2 (11.1)
Bronchitis	1 (5.6)	0	0	0	1 (5.6)
Infective pulmonary exacerbation of CF	1 (5.6)	0	0	0	1 (5.6)
Rhinitis	1 (5.6)	0	0	0	1 (5.6)
Metabolism and nutrition dis.					
Hyperkalaemia	1 (5.6)	0	0	0	1 (5.6)
Musculoskeletal and connective tissue dis.					
Myalgia	1 (5.6)	0	0	0	1 (5.6)
Nervous system dis.					
Headache	0	0	2 (40.0)	1 (20.0)	2 (11.1)
Respiratory, thoracic and mediastinal dis.					
Cough	1 (5.6)	0	0	1 (20.0)	3 (16.7)

Percentages were calculated using total number of patients per treatment group as the denominator.
Admin: administration; AE: adverse event; BI: BI 1265162; BID: twice daily; CF: cystic fibrosis; dis.: disorders; TS: treated set.

Figure legends

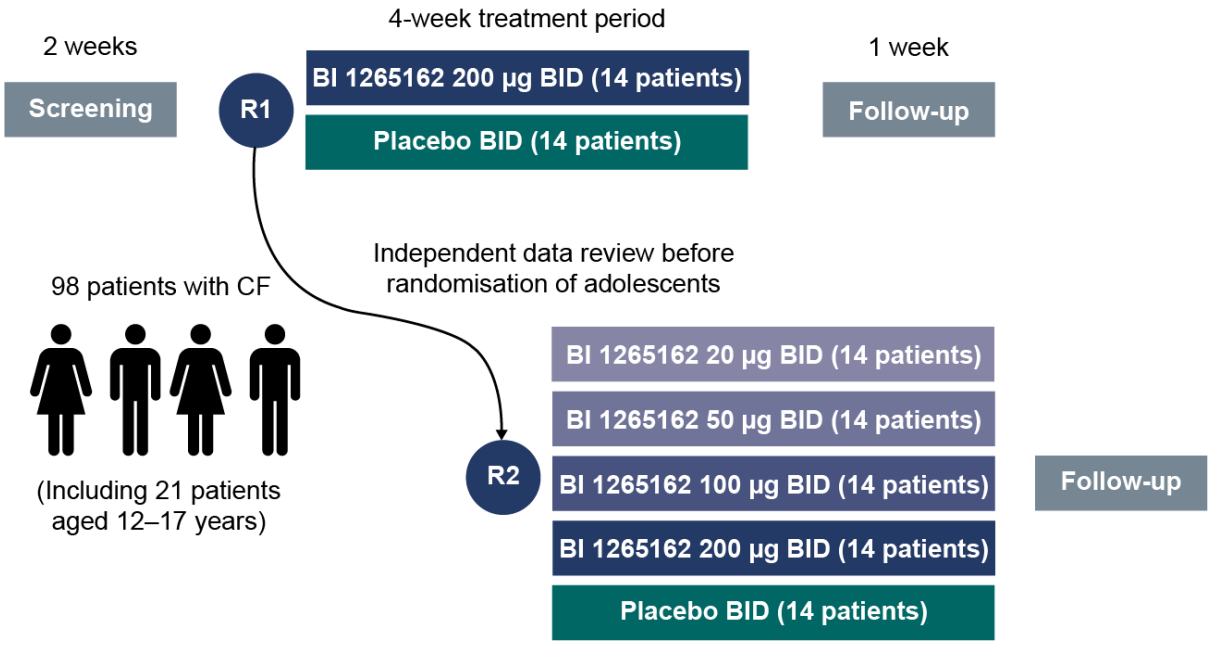

Figure 1. Study design

BID: twice daily; CF: cystic fibrosis; R: randomisation.

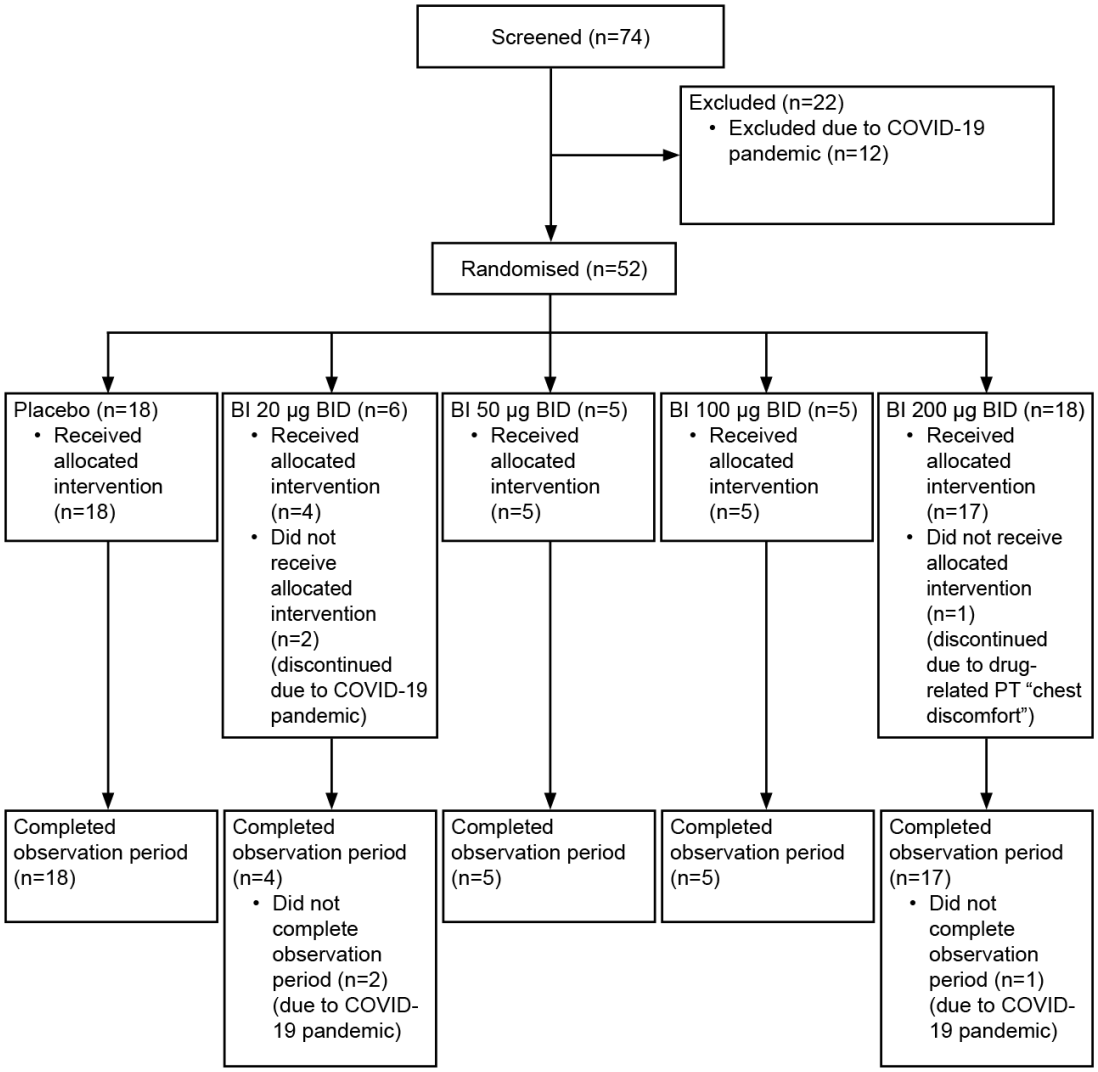

Figure 2. Patient disposition

BI: BI 1265162; BID: twice daily; PT: preferred term.

Efficacy and safety of inhaled ENaC inhibitor BI 1265162 in patients with cystic fibrosis: BALANCE–CF™ 1 – a randomised, Phase II study

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Trial number: NCT04059094

Online supporting information

Methods

Objectives

The objectives of this study were to assess the efficacy, safety and pharmacokinetics of twice-daily (BID) doses of 20 µg, 50 µg, 100 µg and 200 µg BI 1265162, inhaled using the Respimat® Soft Mist™ inhaler (SMI), in addition to standard cystic fibrosis (CF) therapies, including cystic fibrosis transmembrane conductance regulator (CFTR) modulators, compared with BID placebo, in patients aged ≥12 years old.

Study design

This study was a multicentre, multinational, randomised, double-blind, placebo-controlled, parallel-group, dose-ranging study carried out at 29 sites (26 sites with screened patients) across eight countries [1]. A total of 74 patients were screened (enrolled) by 26 centres in Belgium (2 sites), Canada (2 sites), France (5 sites), Germany (5 sites), Spain (1 site), Sweden (2 sites), the United Kingdom (1 site) and the United States (8 sites). The trial was carried out from 24 September 2019 to 24 April 2020.

The patient population in this study was exclusively male. Because this was the first Phase II study performed with this molecule and embryo-foetal development data were not available at study start, Women of Childbearing Potential (WoCBP) were excluded in the initial protocol. Availability of embryo-foetal development data then allowed inclusion of WoCBP using adequate contraception. This was reflected in a

revision of the clinical trial protocol on 18 November 2019, which needed to undergo local regulatory approval processes before implementation.

The study consisted of a 2-week screening period, a 4-week randomised treatment period and a 7-day follow-up period (Figure 1). As the investigational drug was assessed as an add-on therapy to standard of care, patients remained on a stable CF medication regimen (with the exception of bronchodilators, which were withheld prior to lung function testing) from 4 weeks prior to randomisation until the end of the treatment period. Concomitant use of CFTR modulators was allowed, if stable.

A stable medication regimen is defined as the current medication regimen for CF that the patient has been following for at least 4 weeks before Day 1 (randomisation).

A total of 98 patients, starting with adults with the possibility to extend to also include adolescents (from 12 years of age), were planned for randomisation. Twenty-eight patients were first allocated to the highest dose of BI 1265162 (200 µg BID) or placebo BID in a 1:1 ratio (n=14 per group). Once the first 28 patients were randomised, the remaining 70 patients were to be allocated to one of the five treatment arms (200 µg, 100 µg, 50 µg, 20 µg or placebo BID) in a 1:1:1:1:1 ratio, to result in a final ratio of 2:1:1:1:2.

The sample size calculation was based on the following assumptions:

- the primary endpoint (i.e. change from baseline in percent predicted trough forced expiratory volume in 1 second [ppFEV$_1$] at Week 4) was normally distributed
- sided significance level $\alpha = 5\%$
- mean treatment difference (for the highest dose of BI 1265162 vs placebo) was 6% (for interim futility analysis only)

- true maximum treatment effect size of BI 1265162 versus placebo was 6%
- standard deviation was 8% [2]
- pre-specified candidate models.

Randomisation to treatment groups was performed by ALMAC Clinical Technologies Services, United States using an Interactive Voice/Web Response System (IXRS), assigning the appropriated medication number based on the treatment sequence. The randomisation code was generated using a validated system and verified by a trial-independent statistician. The randomisation scheme was provided by Boehringer Ingelheim. A block size of 4 was used for the first 28 adult patients. For the remaining adult patients, a block size of 13 was used. For adolescents, a block size of 7 was to be used.

Patients, investigators, and everyone involved in trial conduct or analysis were to remain blinded with regard to the randomised treatment assignments until after database lock. All treatments were inhaled via the Respimat® SMI. Medications were dispensed by the investigator, study coordinator or pharmacist, depending on the site structure.

The start of adolescent patients' enrolment was to be based on review of adult safety data, carried out by an independent data monitoring committee (DMC) in collaboration with the Cystic Fibrosis Foundation, after every seven patients had completed the treatment period.

An interim futility analysis was planned to be conducted on the first 28 patients to assess efficacy and to prevent exposure of further patients in case of insufficient efficacy. Per protocol, recruitment was to continue during preparation and conduct of

the interim analysis. A decision on discontinuation at the interim analysis was to be made using the following stopping rule:

> Increase in trough percent predicted forced expiratory volume in 1 second ($ppFEV_1$) % <1.5% AND decrease (improvement) in lung clearance index (LCI) <0.3 units.

These criteria were based on the opinion that a clinically meaningful improvement in $ppFEV_1$ or LCI would not be realistically expected to reach an effect considered clinically meaningful should the study fully recruit, i.e. a futility analysis.

Key inclusion and exclusion criteria

- Inclusion criteria:
  - Male or female, aged ≥12 years at screening.
  - Diagnosis of CF (positive sweat chloride ≥60 mEq/L, or genotype with two identifiable mutations and ≥1 clinical phenotypic feature of CF).
  - FEV_1 40–90% predicted at screening and pre-dose at Visit 2 (according to Global Lung Initiative).
- Exclusion criteria:
  - Acute upper or lower respiratory tract infection ≤4 weeks prior to randomisation.
  - Pulmonary exacerbation requiring the use of antibiotics or oral corticosteroids ≤4 weeks prior to randomisation.

- Women of childbearing potential on inadequate contraception (subject to protocol amendment).
- Starting a new chronic medication for CF within 4 weeks of randomisation.

Endpoints

The primary endpoint was the change from baseline in trough (30 minutes prior to dosing) ppFEV$_1$ after 4 weeks of treatment.

Secondary endpoints were:

- Change from baseline in LCI as assessed by N_2 multiple breath washout (N_2MBW) test after 4 weeks of treatment (patients qualified for N_2MBW test if they had a FEV$_1$ >60% of predicted values at screening and were able to complete the N_2MBW test at Visit 2).
- Change from baseline in Cystic Fibrosis Questionnaire – Revised (CFQ-R) [3] total score after 4 weeks of treatment.
- Change from baseline in Cough and Sputum Assessment Questionnaire (CASA-Q©) after 4 weeks of treatment [4].
- Percentage of patients with treatment-emergent adverse events (AEs) up to Day 36, clinical laboratory assessments, vital signs, electrocardiograms, physical examination and chest examination.
- Maximum measured concentration of the analyte in plasma following dose N up to Day 36.
- Pre-dose concentration measured for dose N up to Day 29.

- Area under the concentration−time curve of the analyte in plasma until t hours after dose N up to Day 36.

Treatment compliance

The extent of patient compliance (percentage of prescribed Respimat actuations taken) was measured by the use of a diary, dispensed to patients at Visit 2, with compliance checks and checks of diaries at Visits 3 and 4. A compliance of 80–120% was required.

Statistical analyses

The planned analyses for proof of concept and dose-finding were to use multiple comparison and modelling techniques to measure the difference between the placebo and BI 1265162 treatment groups. Due to a halt in recruitment because of the COVID-19 pandemic followed by discontinuation based on the interim futility results, sample size was limited for the final analysis. Power calculations for the final analysis were based on having $ppFEV_1$ results for at least 24 evaluable patients each for the BI 1265162 200 µg and placebo BID treatment groups and at least 12 evaluable patients each for all other treatment groups; however, the treated set after study termination, which was used for analysis of the primary endpoint, included only 18 patients each in the BI 200 µg and placebo treatment groups and 5 or 6 patients each in the other treatment groups.

To assess the change from baseline in trough $ppFEV_1$ after 4 weeks of treatment, a restricted maximum likelihood-based approach using a mixed model with repeated

measurements (MMRM) was carried out. The analysis included the fixed, categorical effects of treatment at each visit (baseline, Week 1, Week 4), age and the fixed continuous effects of baseline at each visit. Visits were treated as the repeated measure with an unstructured covariance structure used to model the within-patient measurements.

Change from baseline in LCI after 4 weeks of treatment was analysed by covariance (ANCOVA), with adjustment for categorical effects of treatment and the fixed continuous effect of baseline. CASA-Q® analysis was descriptive in nature, with scores described by treatment and domain score for baseline, Day 8, Day 29 and change from baseline. CFQ-R analysis was also descriptive in nature, with total and domain scores for baseline, Day 29 and change from baseline described separately for each treatment group.

Sensitivity analyses

Sensitivity analyses were pre-specified and permitted by the study statistical analysis plan to address any outlier data points and expected variability. Data were reviewed by an Interim Analysis Assessment Committee (Boehringer Ingelheim, independent from the study team) at the interim futility analysis for the impact of outliers.

Three sensitivity analyses were carried out for both $ppFEV_1$ and LCI endpoints. In the first analysis, the same MMRM and ANCOVA models described above were used for ppFEV1 and LCI outcomes, respectively. Individual patient data were excluded based on the following criteria: AEs that could have affected lung function, low (<80%) treatment compliance and unacceptable pulmonary function test quality. These criteria, examined during a blinded medical review, were discussed and

identified in advance. The assumption was that the impact of these criteria on the overall changes in $ppFEV_1$ could be greater than the individual treatment effect size in the 4-week treatment duration (only Week 1 and Week 4 with lung function measurements). After data were reviewed by an Interim Analysis Assessment Committee at the interim futility analysis, a second analysis, a *post hoc* (after unblinding) quantile regression, was carried out to measure median change from baseline to Week 4 in $ppFEV_1$ and LCI in the BI 1265162 200 µg BID and placebo treatment groups. The third analysis was a combination of analyses 1 and 2, whereby a *post hoc* (after unblinding) quantile regression was carried out and patient data were excluded as described above.

Subgroup analysis

A model-based predefined subgroup analysis was performed to investigate any impact of patient characteristics, CFTR mutation status and concomitant CF therapy use on the change from baseline in trough $ppFEV_1$ after 4 weeks of treatment.

Ethics

The protocol was reviewed and approved by the respective Institutional Review Boards/Independent Ethics Committees of the participating centres. The study was carried out in accordance with the principles of the Declaration of Helsinki, in accordance with the International Conference on Harmonisation-Good Clinical Practice (ICH GCP), and in accordance with applicable regulatory requirements.

Prior to patient participation in the study, written informed consent was obtained from each patient (or the patient's legally accepted representative) according to ICH GCP and to the regulatory and legal requirements of the participating country.

Funding of study

The study was funded by Boehringer Ingelheim.

Results

Supplementary tables

Supplementary Table 1. Patients with visit data exclusions in the sensitivity analyses – TS

Treatment group	Patient	Reason	Visit	PT/comment
Placebo	1250008002	AE[1]	Week 1 Week 4	Bronchial congestion
	1250008003	AE[1]	Week 4	Bronchial infection
	1840004001	AE[1]	Week 4	Reactive airway disease exacerbation
	1840004002	AE[1]	Week 4	Cough increased and sputum increased
BI 200 µg	1250008001	AE[1]	Week 4	Spastic bronchial infection
	1276002003	AE[1]	Week 4	Lung infection with *Prevotella melaninogenica*
	1840008002	AE[1]	Baseline Week 1 Week 4	Lung congestion
	1250010002	Insufficient treatment compliance[2]	Week 4	Overall treatment compliance 70%
	1840006001	Unacceptable pulmonary test quality[3]	Baseline Week 1 Week 4	Pre-existing tracheomalacia

[1]Ongoing AE potentially affecting lung function.
[2]Insufficient treatment compliance was defined as compliance <80%.
[3]Unacceptable pulmonary function test quality at baseline and baseline condition considered as important protocol deviation.
AE: adverse event; BI: BI 1265162; PT; preferred term; TS: treated set.

Supplementary Table 2. Summary of baseline and changes from baseline after 4 weeks of treatment in CFQ-R total and Respiratory Domain scores (descriptive statistics) – TS

	CFQ-R total score							CFQ-R Respiratory Domain score						
	Baseline score				Change from baseline after 4 weeks			Baseline score				Change from baseline after 4 weeks		
	N	Mean	SD		N	Mean	SD	N	Mean	SD		N	Mean	SD
Placebo	18	886.296	163.999		18	5.941	79.669	18	69.444	21.495		18	–2.778	18.597
BI 20 µg	6	913.704	112.614		4	27.083	61.626	6	75.000	24.024		4	6.944	5.319
BI 50 µg	5	991.556	124.086		5	11.167	33.968	5	70.000	11.520		5	–2.222	10.092
BI 100 µg	5	878.333	298.360		5	–15.611	62.167	5	61.111	31.427		5	6.667	13.264
BI 200 µg	17	891.797	128.464		16	24.236	58.290	17	63.399	16.204		16	6.597	14.937

BI: BI 1265162; CFQ-R: Cystic Fibrosis Questionnaire – Revised; SD: standard deviation; TS: treated set.

Supplementary Table 3. Summary of baseline and changes from baseline after 4 weeks of treatment in the four separate subscores of the CASA-Q© (descriptive statistics) – TS

	N	Mean	(SD)	N	Mean	(SD)
Cough Symptom Domain Score						
Placebo	18	57.870	(22.592)	18	4.167	(18.798)
BI 20 µg	6	65.278	(24.954)	4	10.417	(17.180)
BI 50 µg	5	56.667	(19.896)	5	8.333	(8.333)
BI 100 µg	5	56.667	(31.402)	5	3.333	(24.008)
BI 200 µg	18	54.167	(22.002)	17	5.392	(15.574)
Cough Impact Domain Score						
Placebo	18	83.681	(15.104)	18	–0.521	(16.648)
BI 20 µg	6	90.625	(6.555)	4	–6.250	(12.758)
BI 50 µg	5	85.000	(16.741)	5	–0.625	(12.771)
BI 100 µg	5	76.875	(32.067)	5	1.250	(14.757)
BI 200 µg	18	84.722	(12.768)	17	0.735	(11.187)
Sputum Symptom Domain Score						
Placebo	18	55.556	(17.620)	18	5.093	(15.950)
BI 20 µg	6	65.278	(25.504)	4	4.167	(8.333)
BI 50 µg	5	60.000	(27.259)	5	10.000	(14.907)
BI 100 µg	5	63.333	(13.944)	5	3.333	(16.245)
BI 200 µg	18	61.574	(17.419)	17	5.392	(19.530)
Sputum Impact Domain Score						
Placebo	18	83.102	(12.250)	18	–0.694	(16.497)
BI 20 µg	6	90.278	(10.092)	4	–4.167	(3.402)
BI 50 µg	5	82.500	(23.459)	5	5.000	(11.562)
BI 100 µg	5	81.667	(36.515)	5	–0.833	(13.944)
BI 200 µg	18	86.343	(11.236)	17	0.490	(11.110)

BI, BI 1265162; CASA-Q©: Cough and Sputum Assessment Questionnaire; SD: standard deviation; TS: treated set.

Supplementary Table 4. Pharmacokinetic parameters (N, gMean, gCV) of twice-daily BI 1265162 by treatment group – PKS

	BI 20 µg				BI 50 µg			BI 100 µg				BI 200 µg		
	N	gMean[1]	gCV[2]		N	gMean[1]	gCV[2]	N	gMean[1]	gCV[2]		N	gMean[1]	gCV[2]
$C_{0.083}$	4	131	106		2	-	-	4	732	12.0		16	1000	106
$C_{0.083,ss,15}$	3	207	59.9		4	471	30.0	4	1010	20.1		16	1110	84.8
$C_{0.083,ss,57}$	3	162	76.3		3	463	15.4	5	573	94.0		14	1080	165
$C_{pre,ss,15}$	3	7.82	28.0		4	24.3	31.8	5	38.4	292		14	43.8	95.6
$C_{pre,ss,57}$	1	-	-		3	13.0	80.8	3	22.3	48.3		12	37.2	56.9
$C_{max,ss,15}$	5	163	57.0		4	471	30.0	4	1010	20.1		17	1120	81.5
$AUC_{0-4,ss,15}$	5	192	45.3		4	541	19.1	4	1020	8.93		17	1380	71.0

[1]Units were pmol/L for $C_{0.083}$, $C_{0.083,ss,15}$, $C_{0.083,ss,57}$, $C_{pre,ss,15}$, $C_{pre,ss,57}$, $C_{max,ss,15}$ and h×pmol/L for $AUC_{0-4,ss,15}$.

[2]Units were % for gCV.

$AUC_{0-4,ss,15}$: area under the curve from 0 to 4 h at steady state after dose 15; BI: BI 1265162; $C_{0.083}$:concentration at time 0.083 h; $C_{0.083,ss,15}$: concentration at time 0.083 h at steady state after dose 15; $C_{0.083,ss,57}$: concentration at time 0.083 h at steady state after dose 57; $C_{pre,ss,15}$: pre-dose concentration at steady state for dose 15; $C_{pre,ss,57}$: pre-dose concentration at steady state for dose 57; $C_{max,ss,15}$: maximum measured concentration at steady state following dose 15; gCV: geometric coefficient of variation; gMean: geometric mean; PKS: pharmacokinetics set.

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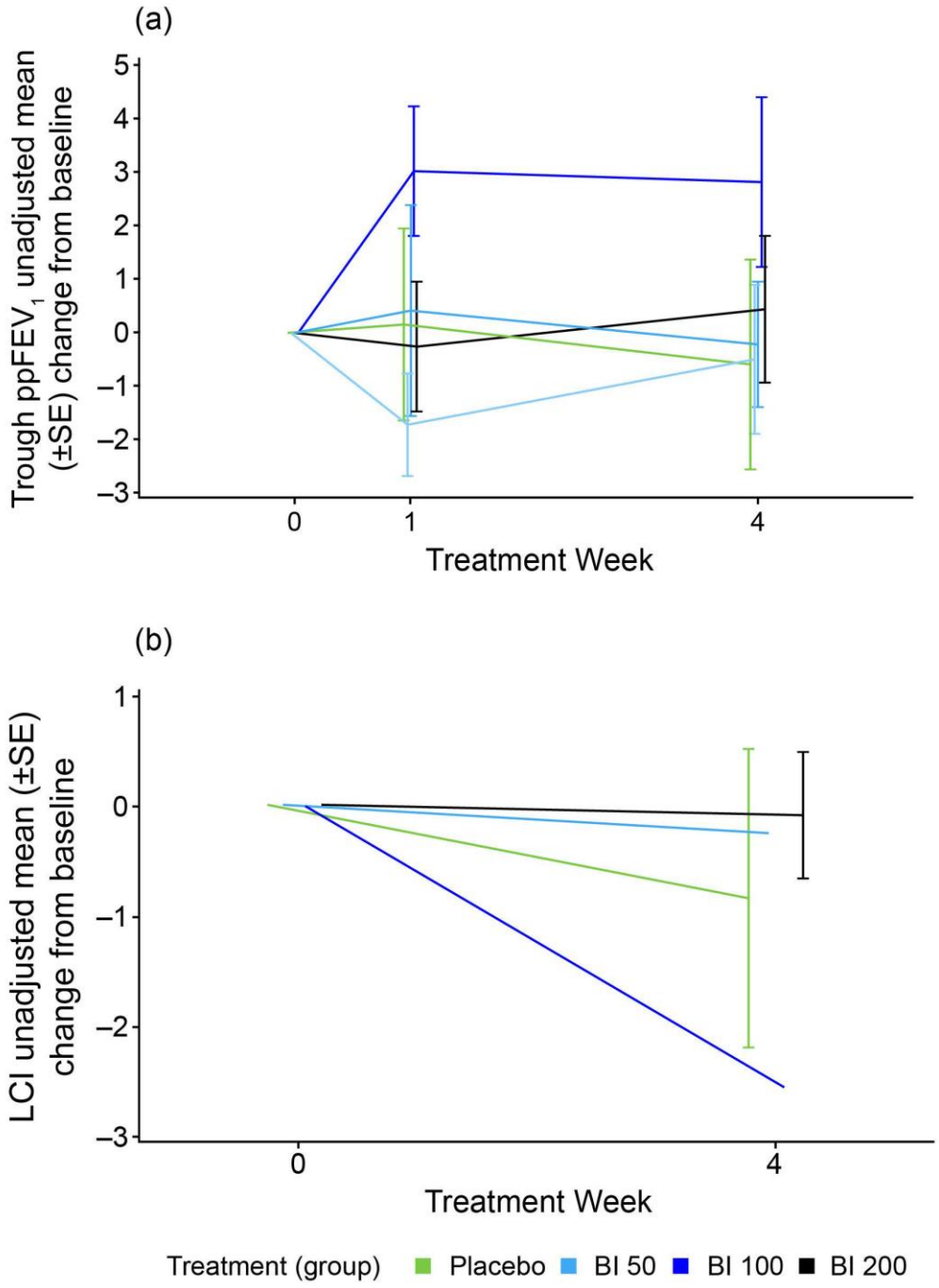
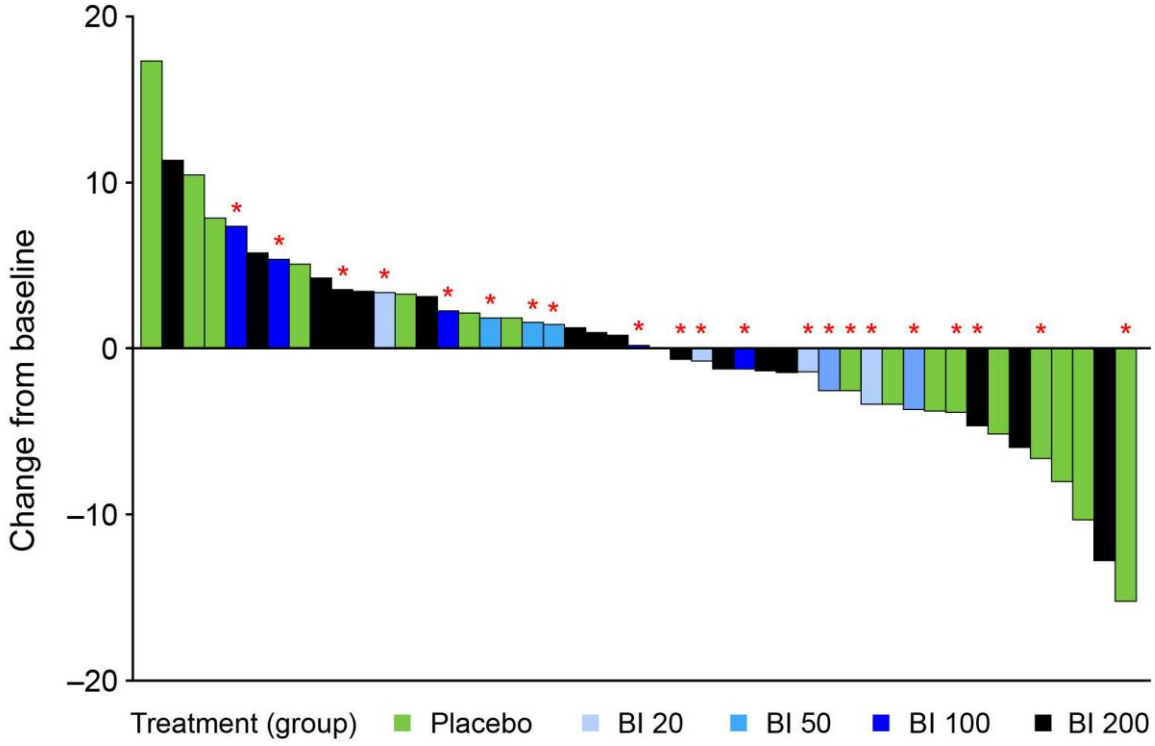
Supplementary figure legends



Supplementary Figure 1. (a) Change from baseline in unadjusted mean (±SE) trough ppFEV$_1$ at Weeks 1 and 4 of treatment – TS and (b) LCI after 4 weeks of treatment – N$_2$MBWS

No patients in the BI 1265162 20 µg BID treatment group were included in the N$_2$MBWS. The BI 1265162 50 µg and 100 µg BID treatment groups only had one

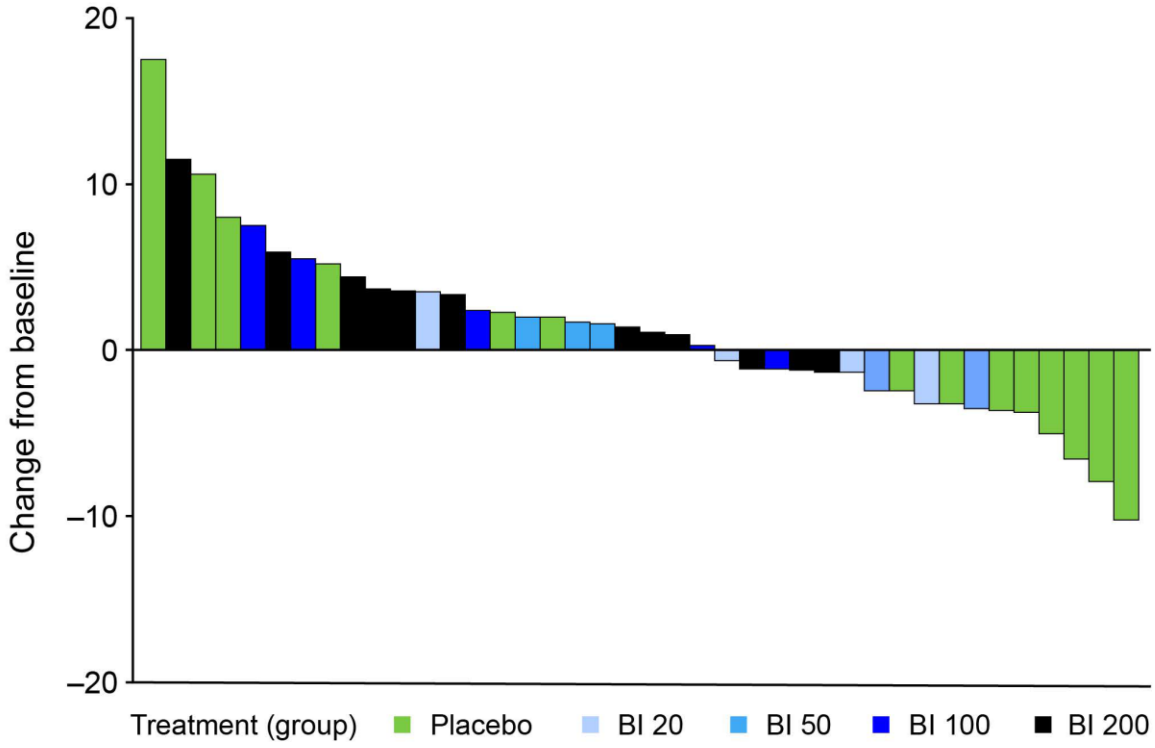
patient each included in the N_2MBWS; accordingly, no standard error could be calculated for these groups. BI: BI 1265162; BID: twice daily; LCI: lung clearance index; N_2MBWS: N_2 multiple breath washout set; ppFEV$_1$: percent predicted forced expiratory volume in 1 second; SE: standard error; TS: treated set.



Supplementary Figure 2. Change from baseline in ppFEV$_1$ after 4 weeks of treatment for individual patients, all dose groups – TS

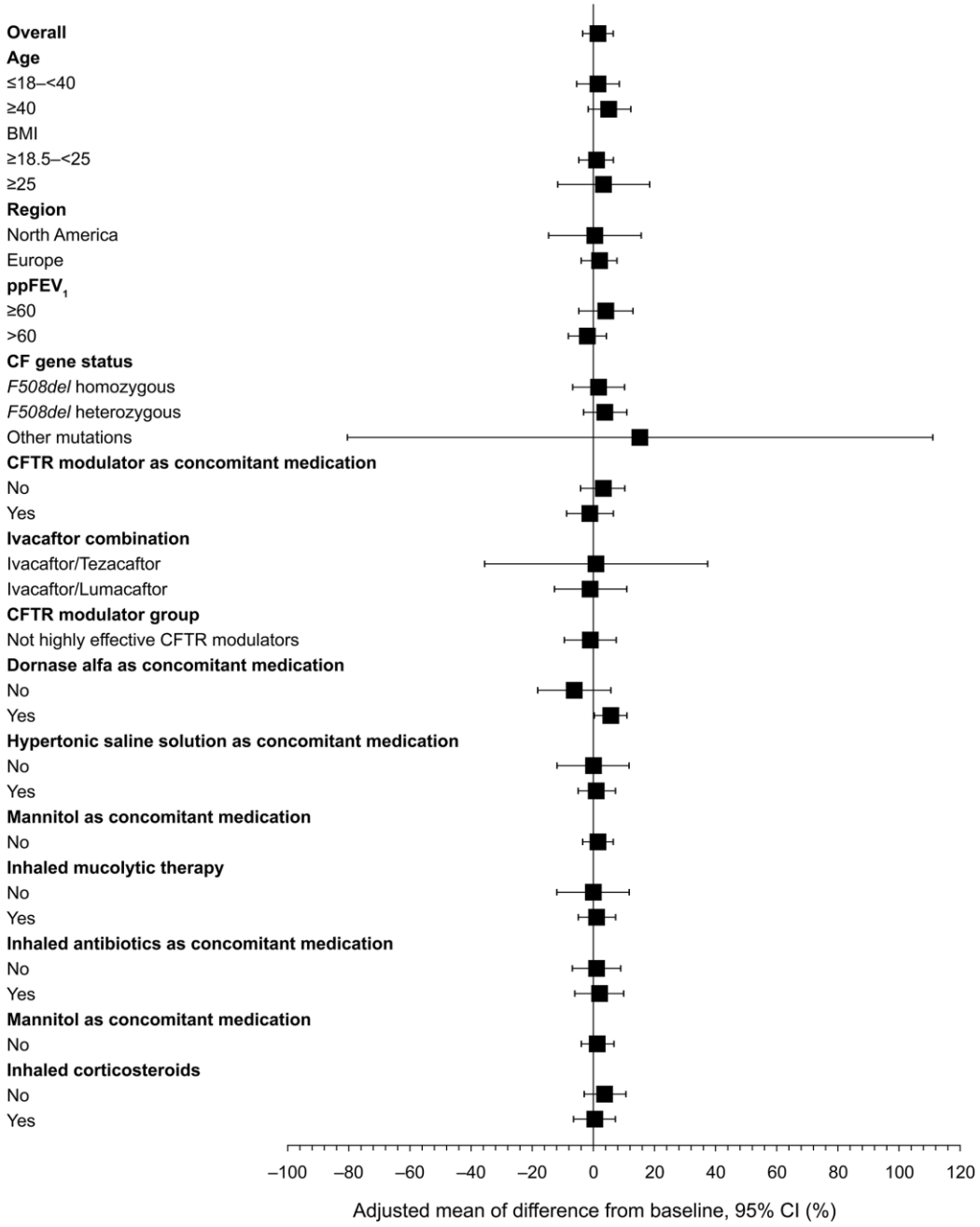
* Additional patient visit data added after interim analysis.

BI: BI 1265162; MMRM: mixed model for repeated measures; ppFEV$_1$: percent predicted forced expiratory volume in 1 second; TS: treated set.



Supplementary Figure 3. Sensitivity analysis for change from baseline in ppFEV$_1$ after 4 weeks of treatment for individual patients (MMRM) – TS

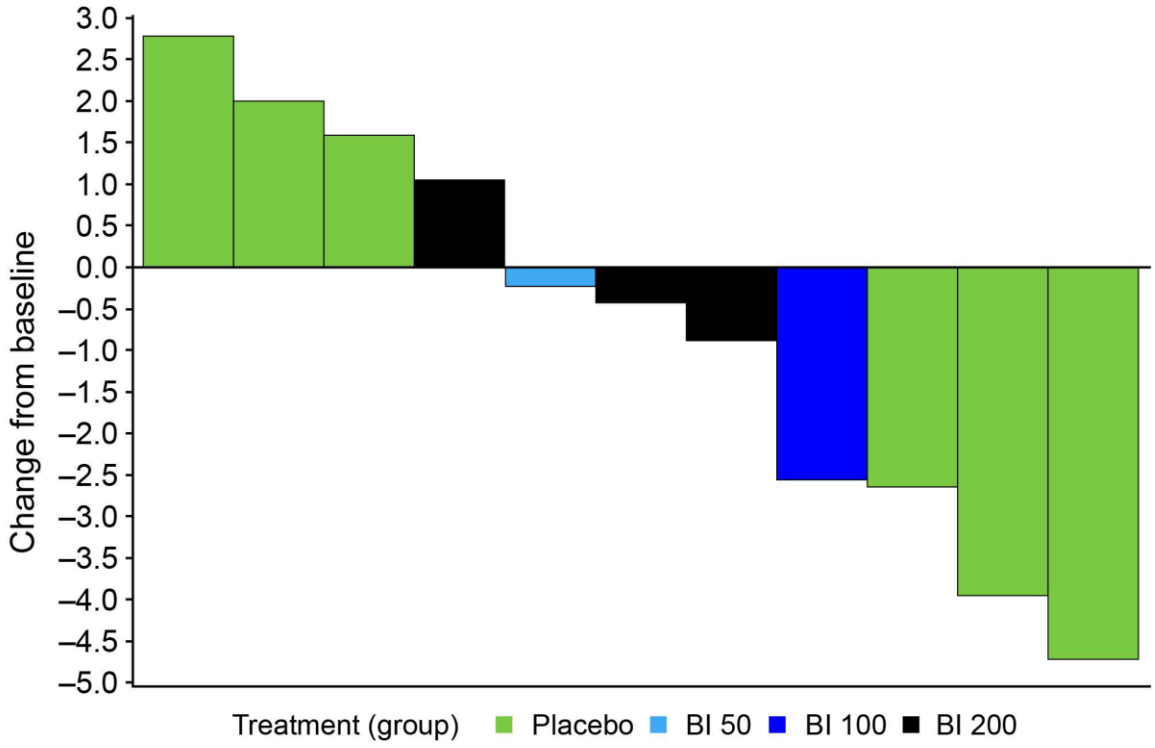
Data from visits were excluded based on AEs that might have affected pulmonary function tests, compliance, and unacceptable pulmonary function test quality at baseline and/or baseline condition considered as important protocol deviation. AE: adverse event; BI: BI 1265162; MMRM: mixed model for repeated measures; ppFEV$_1$: percent predicted forced expiratory volume in 1 second; TS: treated set.



Supplementary Figure 4. ppFEV$_1$ subgroup analyses (MMRM) of difference between BI 1265162 and placebo from baseline – TS

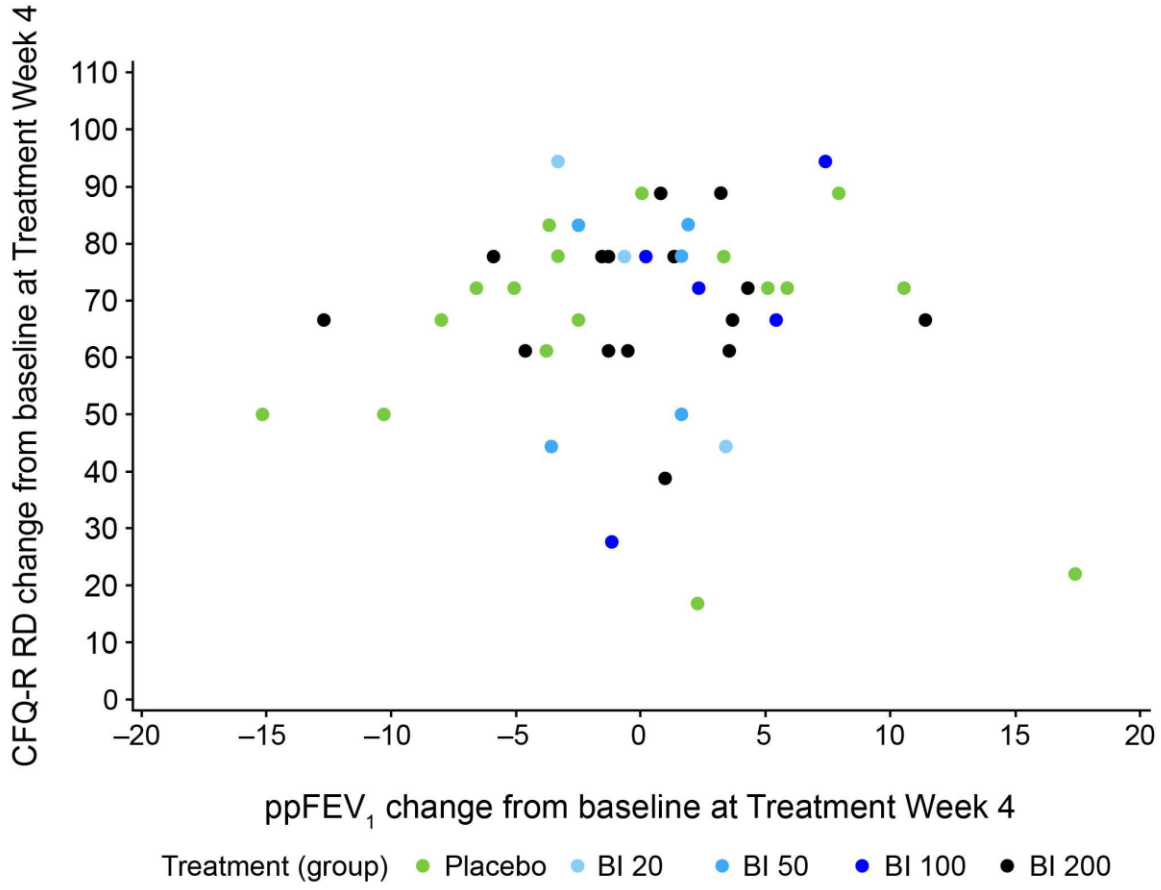
BMI: body mass index; CF: cystic fibrosis; CFTR: cystic fibrosis transmembrane conductance regulator; CI: confidence interval; MMRM: mixed model for repeated

measures; ppFEV$_1$: percent predicted forced expiratory volume in 1 second; TS: treated set.



Supplementary Figure 5. Change from baseline in LCI after 4 weeks of treatment for individual patients, final analysis – N_2MBWS

BI: BI 1265162; LCI: lung clearance index; N_2MBWS: N_2 multiple breath washout set.



Supplementary Figure 6. Correlation analysis between change in CFQ-R Respiratory Domain score and change in $ppFEV_1$ after 4 weeks of treatment

BI: BI 1265162; CFQ-R RD: Cystic Fibrosis Questionnaire – Revised Respiratory Domains; $ppFEV_1$: percent predicted forced expiratory volume in 1 second.

References

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