First clinical trials of the inhaled epithelial sodium channel inhibitor BI 1265162 in healthy volunteers

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ABSTRACT

Background: Inhibition of the epithelial sodium channel (ENaC) represents a mutation-agnostic therapeutic approach to restore airway surface liquid hydration and mucociliary clearance in patients with cystic fibrosis. BI 1265162 is an inhaled ENaC inhibitor with demonstrated preclinical efficacy.

Methods: Three phase I trials of BI 1265162 in healthy male subjects are presented: NCT03349723 (single-rising-dose trial evaluating safety, tolerability and pharmacokinetics (PK)); NCT03576144 (multiple-rising-dose trial evaluating safety, tolerability and PK); and NCT03907280 (absolute bioavailability trial).

Results: BI 1265162 single doses ⩽1200 µg and multiple doses of 600 µg were well tolerated. Adverse events were balanced across treatment groups, were of mainly mild or moderate intensity and resolved by trial-end. One subject discontinued from trial medication on day 7 (asymptomatic hyperkalaemia adverse event; recovered day 8). One subject experienced a serious adverse event (neuropathia vestibularis) leading to hospitalisation and missed one of the four dosing periods. Both events were not considered to be drug-related and subjects recovered. BI 1265162 displayed dose-proportional, time-independent PK; maximum accumulation was 1.6-fold; calculated effective elimination half-life was 3.6–8.7 h over the dose ranges tested. Renal excretion was not a major drug elimination route. Oral and inhaled dosing (±activated oral charcoal) absolute bioavailability was 0.50% and ∼40%, respectively.

Conclusion: BI 1265162 single or multiple doses up to 6.5 days were well tolerated. Systemic exposures mainly represent drug absorbed through the lungs and not the gastrointestinal tract, with ∼40% of the inhaled dose reaching the systemic circulation. Accumulation was minimal. Twice-daily dosing is supported for future development.

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Cell and animal studies have demonstrated that BI 1265162 is a potent ENaC inhibitor. Three phase I trials show that single- and multiple-dose BI 1265162 is safe. BI 1265162 is being tested in phase II studies, using twice-daily dosing, in people with CF. https://bit.ly/3nPUkrO

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Introduction

Cystic fibrosis (CF) is a multisystem disorder caused by mutations in the cystic fibrosis transmembrane conductance regulator gene (CFTR). This can lead to defects in the CFTR protein [1], causing aberrant chloride transport in epithelial tissues resulting in alterations to the hydration and pH of airway surface liquid (ASL) [2, 3].

Over the past decade, significant clinical progress has been made to directly target CFTR mutations to increase the quantity and/or enhance the function of the protein [3]. However, even with the arrival of the new triple CFTR modulator Trikafta™ (ivacaftor, tezacaftor, elexacaftor), which is suitable for patients with at least one F508del mutation, ⩾10% of patients with CF still remain untreated due to unsuitable genotype [4, 5]. Furthermore, CFTR modulators restore 10–50% of CFTR function [6–8] and patients may continue to have exacerbations [4]. Therefore, alternative or additional approaches to therapy, including modulating alternative targets that compensate for CFTR dysfunction, are needed beyond CFTR modulation [2].

One such target is the epithelial sodium channel (ENaC). ENaC is expressed in the conducting airways, alveolar airspaces and the distal colon, and highly expressed in the cortical collecting duct of the kidney. Through sodium and water resorption, and chloride secretion, ENaC and CFTR together maintain a finely tuned homeostatic mechanism to keep ASL hydrated and allow mucociliary clearance, needed for a sterile lung environment [9, 10]. ENaC is hyperactivated in CF and leads to increased absorbance of sodium ions [11–13] and water resorption from the epithelial luminal surface, causing dehydrated mucus and compressed cilia, resulting in poor mucociliary clearance [14].

ENaC inhibition represents a mutation-agnostic therapeutic approach [9] to restore ASL hydration and enhance mucociliary clearance in people with CF. The potential therapeutic value of ENaC inhibition is supported by the observation of slow lung disease progression in CF patients with a mutation in the δ-subunit of ENaC that causes reduced ENaC activity [15]. In addition to the mutation-agnostic property of an ENaC inhibitor, a synergistic effect with CFTR modulators is expected [9]. Indeed, ENaC inhibition further reduced transepithelial water absorption in both CF and normal airway cultures treated with CFTR modulators [16].

No previous potential ENaC inhibitor therapy has succeeded in clinical trials, probably due to lack of potency, inadequate dosing and/or deposition by inhalation in patients with chronic CF lung disease, induced hyperkalaemia, short study duration, non-study-related exacerbations or lack of end-point sensitivity [17]. Historically, small-molecule direct inhibitors have formed the large part of the clinical development programme. Amiloride failed phase II studies due to efficacy issues [18], and the second- and third-generation amiloride derivatives benzamil [19] and GS-9411 [20] failed preclinical and phase I studies, respectively, due to efficacy issues and hyperkalaemia, respectively. BI 443651 failed phase I due to palatability issues (NCT02706925). VX-731, in combination with the CFTR modulator ivacaftor/lumacaftor, failed phase II (NCT02709109). Likewise, QBW276 was terminated during phase II for strategic reasons (NCT02566044). Camostat, a small-molecule inhibitor of prostatin (a channel-activating protease that is a major regulator of ENaC activity), failed in phase II due to adverse events [21]. Outside of small-molecule inhibitors, the SPLUNC-1 peptide analogue SPX-101 failed phase II due to lack of efficacy (NCT03229252).

BI 1265162 is a small-molecule direct ENaC inhibitor. In rat and sheep studies, no effects on serum potassium and plasma electrolytes were observed, and BI 1265162 demonstrated a markedly higher efficacy than amiloride (a 30–70-fold lower median inhibitory concentration) [16]. Importantly, these studies also demonstrated that the efficacy of BI 1265162 is mutation-agnostic and synergistic with the

TABLE 1 Baseline characteristics of all three trials			
	SRD trial	**MRD trial**	**Bioavailability trial**
Subjects	56	50	12
Age years	34.7±8.8 (21–51)	32.8±6.6 (21–45)	36.8±9.5 (23–49)
Race			
White	55 (98.2)	48 (96.0)	12 (100)
Black/African American	1 (1.8)	1 (2.0)	0
Asian	0	1 (2.0)	0
Hispanic/Latino			
No	55 (98.2)	50 (100)	12 (100)
Yes	1 (1.8)	0	0
BMI kg·m^{-2}	26.8±2.3	24.6±2.8	25.1±2.6

Data are presented as n, mean±SD (range) or n (%). SRD: single-rising-dose; MRD: multiple-rising-dose; BMI: body mass index.

CFTR modulator lumacaftor/ivacaftor [16]. Data from three phase I trials with BI 1265162 in healthy male subjects are presented: 1) a first-in-man, single-rising-dose (SRD) trial evaluating safety, tolerability and pharmacokinetics (PK) of inhaled BI 1265162 (NCT03349723); 2) a multiple-rising-dose (MRD) trial evaluating safety, tolerability and PK of inhaled BI 1265162 (NCT03576144); and 3) a trial determining absolute bioavailability of BI 1265162 following oral and inhaled (±activated charcoal) administration (NCT03907280).

Methods

A brief summary of methods is provided. For detailed methodology, see the supplementary material.

All three trials enrolled healthy, nonsmoking males aged 18–50 years (with the exception of the MRD trial: age 18–45 years), with a body mass index of 18.5–29.9 kg·m^{-2} and normal lung function (forced expiratory volume in 1 s and forced vital capacity ⩾80% predicted normal). Inhaled doses of BI 1265162 were administered *via* the Respimat Soft® Mist™ inhaler [22]. The trials were carried out sequentially in the order SRD, MRD, bioavailability trial. For the bioavailability trial, lung function was not part of eligibility criteria (table 1). Serum and urine electrolytes in the SRD and MRD trials were closely monitored to mitigate risk of renal ENaC inhibition, although systemic exposure of BI 1265162 was not expected to reach relevant levels as is observed with orally administered amiloride (an ENaC inhibitor in clinical use as a potassium-sparing diuretic). Changes in serum potassium were monitored as part of dose-escalation criteria in the SRD and MRD trials.

NCT03349723: safety and PK of BI 1265162 in an SRD trial

This was a single-centre, partially randomised, single-blind, placebo-controlled trial to investigate the safety and tolerability of BI 1265162 following inhaled administration of SRDs (3 µg, 10 µg, 30 µg, 100 µg, 300 µg, 600 µg, 1200 µg) (figure 1a). The secondary objective was the exploration of PK. Plasma samples were taken up to 48 h post-dose, with the exception of the 1200 µg group, where samples were taken up to 72 h post-dose. Safety, PK parameters and further end-points are detailed in table 2. Descriptive statistics were calculated for all end-points. Palatability and acceptability of inhaled BI 1265162 were assessed 15 min post-dosing using a multiple-choice questionnaire.

NCT03576144: safety and PK of BI 1265162 in an MRD trial

This single-centre, randomised, double-blind, placebo-controlled trial investigated the safety and tolerability of BI 1265162 (10 µg, 30 µg, 100 µg, 300 µg, 600 µg) following inhaled administration once daily in the morning of days 1 and 8 and twice daily on days 2–7 (figure 1b). Secondary objectives were PK, including dose proportionality and time dependency, following multiple dosing. Descriptive statistics were calculated for all end-points. For PK parameters, dose proportionality was assessed using a power model. Safety, PK parameters and further end-points are detailed in table 2.

NCT03907280: bioavailability of BI 1265162

This was a four-arm, open-label, randomised, single-dose, three-way crossover trial followed by a fixed treatment in healthy male subjects to investigate the absolute bioavailability of BI 1265162 following oral and inhaled administration, ±oral activated charcoal given pre- and post-inhalation, with a 1-h intravenous
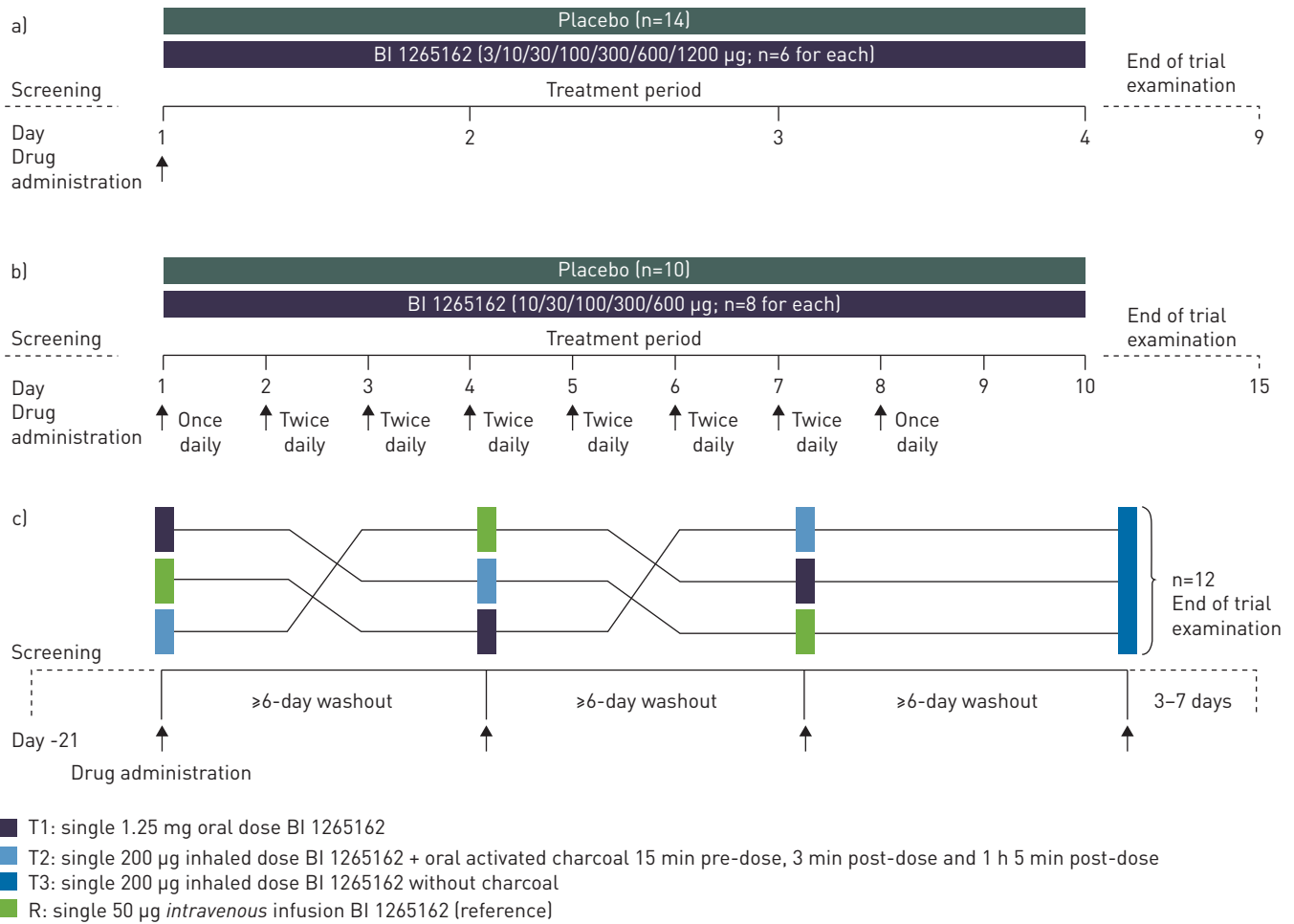
FIGURE 1 Trial design of a) the single-rising-dose trial, b) the multiple-rising-dose trial and c) the bioavailability trial. T: treatment; R: reference.

infusion administered as the reference. Safety and PK parameters are detailed in table 2. Safety and further PK end-points were analysed descriptively.

Single doses of BI 1265162 were evaluated: 1) 1.25 mg oral (T1); 2) 200 µg inhaled with 10 g of activated charcoal given orally pre- and post-dosing (T2); 3) 200 µg inhaled without activated charcoal (T3); and 4) 1-h 50 µg *i.v.* infusion (reference (R)). Subjects were randomly allocated to one of the following treatment sequences: T1-T2-R-T3, R-T1-T2-T3, T2-R-T1-T3 (figure 1c). Each treatment was separated by a ⩾6-day washout period. Patients were followed-up for 3–7 days post-trial-end.

Results

Baseline characteristics and safety data are summarised in tables 1 and 3, respectively.

SRD trial

56 subjects received their assigned single dose of BI 1265162 or placebo and completed the trial according to the clinical trial protocol.

All adverse events were of mild or moderate intensity and resolved by trial-end. Treatment-emergent adverse events (TEAEs) were reported for six (14.3%) out of 42 subjects treated with BI 1265162 and two (14.3%) out of 14 subjects treated with placebo (table 3). Investigator-defined drug-related adverse events occurred in two (4.8%) out of 42 subjects receiving BI 1265162 and none of the 14 subjects receiving placebo. In the BI 1265162 100 µg dose group, one (16.7%) subject reported cough of mild intensity 5 min post-drug administration. In the 300 µg BI 1265162 dose group, one subject (16.7%) had dry eye of mild intensity on the day of drug administration; this adverse event resolved on the next day. No deaths, serious adverse events (SAEs), protocol-specified adverse events of special interest (AESIs) or other significant adverse events were reported in this trial. No other relevant changes were reported in safety laboratory,

TABLE 2 Primary, secondary and further end-points of all three trials				
	Primary end-point(s)	**Secondary end-point(s)**	**Further end-points**	**Further safety parameters of interest**
Single-rising-dose	Drug-related adverse events	C_{max} AUC_{0-1}	t_{max} $AUC_{0-\infty}$ CL/F $t_{1/2}$	Physical examination Safety laboratory tests 12-lead ECG Continuous ECG monitoring Vital signs Spirometry (FEV_1, FVC and FEF_{25-75}) Palatability
Multiple-rising-dose	Drug-related TEAEs			Physical examination Safety laboratory tests Serum and urine electrolytes 12-lead ECG Vital signs Spirometry
First dose		C_{max} AUC_{0-12}	t_{max} $t_{1/2}$ $f_{e_{0-12}}$	
Last dose		$C_{max,ss}$ $AUC_{\tau,ss}$	$t_{max,ss}$ RA_{Cmax} $RA_{AUC0-12}$ $f_{e_{0-12,ss}}$	
Bioavailability	$AUC_{0-\infty}$		C_{max} t_{max}	Physical examination Safety laboratory tests 12-lead ECG Vital signs

C_{max}: maximum measured concentration of BI 1265162 in plasma after single dose; AUC_{0-1}: area under the curve of BI 1265162 in plasma from dosing to 1 h post-dosing; t_{max}: time administered drug excreted from last dosing to the maximum concentration of BI 1265162 in plasma after single dose; $AUC_{0-\infty}$: area under the concentration–time curve of BI 1265162 in plasma over the time interval from 0 extrapolated to infinity; CL/F: apparent clearance; $t_{1/2}$: observed terminal elimination half-life of BI 1265162 in plasma; FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity; FEF_{25-75}: forced expiratory flow between 25% and 75% of the FVC; TEAE: treatment-emergent adverse event; AUC_{0-12}: area under the curve of BI 1265162 in plasma over a 12-h period after single dose; $f_{e_{0-12}}$: fraction of administered drug excreted unchanged in urine over a 12-h period after single dose; $C_{max,ss}$: maximum measured concentration of BI 1265162 in plasma at steady state; $AUC_{\tau,ss}$: area under the curve of BI 1265162 in plasma over a 12-h period at steady state; $t_{max,ss}$: time administered drug excreted from last dosing to the maximum concentration of BI 1265162 in plasma at steady state; RA_{Cmax}: accumulation ratio based on $C_{max,ss}$; RA_{AUC}: accumulation ratio based on AUC_{0-12} and $AUC_{\tau,ss}$; $f_{e_{0-12,ss}}$: fraction of administered drug excreted unchanged in urine over a 12-h period at steady state.

vital signs, 12-lead ECG, continuous ECG or spirometry values. No relevant changes in plasma or urine electrolytes were detected.

To assess palatability and acceptability, a questionnaire was filled in shortly post-dosing. No relationship was found between dose level and palatability/acceptability. Of subjects reporting a taste (40.5%), "bitter" was most frequently reported. 98% of subjects indicated that they would take the medication long term.

MRD trial

50 subjects entered the MRD trial and received trial medication. In each dose group, eight subjects received BI 1265162 and two received placebo. One subject in the BI 1265162 300 µg dose group discontinued from trial medication on day 7 due to hyperkalaemia according to protocol. All other subjects were treated as planned and completed the trial.

All adverse events were of mild or moderate intensity and resolved by trial-end. TEAEs were reported for 14 (35%) out of 40 subjects treated with BI 1265162 and one (10%) out of 10 subjects treated with placebo (table 3). Investigator-defined drug-related adverse events were reported in 11 (27.5%) out of 40 subjects receiving BI 1265162 and none of the 10 subjects receiving placebo. The most frequently reported drug-related TEAE in subjects receiving BI 1265162 was oropharyngeal pain (three (7.5%) out of 40). Diarrhoea, dyspepsia, dizziness and cough were each reported as drug-related TEAEs in two (5.0%) out of 40 subjects receiving BI 1265162.

TABLE 3 Adverse events												
	Placebo	BI 1265162										
		3 µg	10 µg	30 µg	100 µg	300 µg	600 µg	1200 µg	1.25 mg oral solution	200 µg inhaled, with activated charcoal	200 µg inhaled, without activated charcoal	50 µg 1-h intravenous solution
SRD trial												
Subjects	14	6	6	6	6	6	6	6				
Adverse event												
Subjects with any adverse event	2 (14.3)	1 (16.7)	0	1 (16.7)	1 (16.7)	2 (33.3)	0	1 (16.7)				
Headache	1 (7.1)	1 (16.7)	0	1 (16.7)	0	1 (16.7)	0	0				
Nasopharyngitis	1 (7.1)	0	0	0	0	0	0	1 (16.7)				
Cough	0	0	0	0	1 (16.7)	0	0	0				
Dry eye	0	0	0	0	0	1 (16.7)	0	0				
Subjects with drug-related adverse events	0	0	0	0	1 (16.7)	1 (16.7)	0	0				
MRD trial												
Subjects	10		8	8	8	8	8					
Subjects with any adverse event	1 (10.0)		1 (12.5)	2 (25.0)	5 (62.5)	4 (50.0)	2 (25.0)					
Adverse events in ⩾2 subjects												
Headaches	0		1 (12.5)	0	1 (12.5)	0	1 (12.5)					
Diarrhoea	0		0	0	2 (25.0)	1 (12.5)	0					
Oropharyngeal pain	0		0	0	2 (25.0)	0	1 (12.5)					
Cough	0		0	0	0	1 (12.5)	1 (12.5)					
Dyspepsia	0		1 (12.5)	1 (12.5)	0	0	0					
Dizziness	0		0	0	0	2 (25.0)	0					
Subjects with drug-related adverse events	0		1 (12.5)	1 (12.5)	4 (50.0)	4 (50.0)	1 (12.5)					
Subjects with adverse events leading to discontinuation of trial drug	0		0	0	0	1 (12.5)	0					
Bioavailability trial												
Subjects									12	12	12	12
Subjects with any adverse events									2 (16.7)	0	1 (8.3)	1 (8.3)
Subjects with serious adverse events (neuropathia vestibularis right)									1 (8.3)	0	0	0
Subjects with drug-related adverse events									0	0	0	0
Subjects with adverse events leading to discontinuation of trial drug									0	0	0	0

Data are presented as n or n (%). SRD: single-rising-dose; MRD: multiple-rising-dose.

One subject in the 300 µg dose group discontinued from trial drug on day 7 due to hyperkalaemia. A first transient increase of serum potassium was observed on day 3 (5.69 mmol·L^{-1}; normal range 3.30–5.10 mmol·L^{-1}), with recovery the next day to within normal range (4.95 mmol·L^{-1}). Serum potassium was again above the upper limit of normal following the morning dosing on day 6 (5.91 mmol·L^{-1}) and continued above normal on day 7 (5.80 mmol·L^{-1}). Treatment with BI 1265162 was stopped on day 7 and the subject recovered within 1 day (day 8: 4.29 mmol·L^{-1}, 4.35 mmol·L^{-1}; day 9: 4.85 mmol·L^{-1}). The subject was asymptomatic with no clinical or ECG findings, and no change in other serum electrolytes (sodium, chloride, calcium), serum creatinine, urine aldosterone, urine creatinine or urine electrolytes (sodium, potassium, chloride) was observed concomitantly. This subject had the highest PK plasma BI 1265162 concentration in the 300 µg BI 1265162 dose group (3670 pmol·L^{-1}) at 10 min post-dose on day 7; however, this concentration was lower than the highest observed concentration measured at 10 min post-dose in the 600 µg dose group (8560 pmol·L^{-1}). There were no clinically significant changes in electrolyte concentrations observed following 600 µg dosing, with no clinical symptoms or ECG findings. The observed hyperkalaemia in this subject could thus not be explained by the mode of action of ENaC inhibition in the kidney, which should have resulted in changes of the urine electrolytes. In the absence of alternative reasons, the exact reason remains unclear and individual susceptibility of this subject cannot be excluded with the available data.

No other subject had clinically relevant electrolyte changes. No changes or numerical trends were observed by BI 1265162 dose group when compared with placebo for serum electrolytes, urine aldosterone, fractioned urinary creatinine-adjusted sodium or potassium, and for the urinary sodium/potassium ratio, including in the subject showing potassium above the upper limit of normal. No other relevant changes were reported in safety laboratory data, vital signs, 12-lead ECG or spirometry values. No deaths, SAEs or protocol-specific AESIs were reported.

Bioavailability trial

All treatments were well tolerated by the 12 subjects. All TEAEs (oral n=2; inhaled (without charcoal) n=1; *i.v.* n=1) were of mild or moderate intensity, with the exception of one SAE in the BI 1265162 oral group. This subject had participated uneventfully in the *i.v.* group, and in the following oral drug exposure, experienced vomiting and a vestibular disorder (reported term: neuropathia vestibularis), which led to hospitalisation. The SAE started on day 1, lasted 24 days, and was assessed as not related to trial medication. The subject recovered and participated afterwards in an inhaled-treatment group without any adverse events, receiving three out of the four planned doses. All adverse events were resolved by trial-end; no deaths or other SAEs were reported. No clinically relevant changes in safety laboratory parameters, vital signs or plasma and urine electrolytes were detected in this trial.

Pharmacokinetics

The resulting PK parameters from all three clinical trials are summarised in table 4.

Geometric mean (gMean) plasma concentration–time profiles for BI 1265162 in the SRD and MRD trials are shown in figure 2. Following both single and multiple dosing, BI 1265162 was quickly absorbed into the systemic circulation, with a median time from dosing to maximum plasma concentration (C_{max}) of 5–11 min post-dose. Following C_{max}, plasma BI 1265162 concentrations declined in a multi-exponential manner. Following single inhaled doses of ⩾300 µg, a slower elimination phase was observed from ~12 h post-dose, with concentrations at 24 h post-dose ~1% of the respective C_{max} values. Following the highest single dose (1200 µg), where plasma concentrations were measured up to 72 h post-dose, the observed gMean terminal elimination half-life was 15.5 h. For the lower doses, this terminal phase could not be accurately quantified due to the shorter investigational period of 48 h and a number of samples being below the bioanalytical assay's lower limit of quantification (LLOQ).

Following twice-daily dosing, steady state was attained post-second dose. There was no apparent accumulation in C_{max} values over time following 10, 30 and 600 µg twice daily, and a relatively small amount of accumulation in C_{max} values following 100 µg (1.64-fold) and 300 µg (1.35-fold) twice daily. The accumulation in area under the curve over the time interval 0–12 h (AUC_{0-12}) was ~1.6-fold at maximum. The effective elimination half-life, calculated from accumulation and dosing frequency, was calculated to be ~3.6–8.7 h.

Difficulties in capturing the true terminal elimination phase in the SRD, which appeared to start ~12 h post-dose over the full dose range, meant that different phases of the multi-exponential decline were characterised following the different doses. Post-dose, BI 1265162 concentrations were below the assay's LLOQ from ~1 h for the 3 µg dose, 4 h for the 10 µg dose, 6 h for the 30 µg dose and ⩾12 h for doses of ⩾100 µg. Thus, in order to compare the same portion of the concentration–time curve over the full dose range to assess dose proportionality, AUC 0–1 h (AUC_{0-1}) was used instead of $AUC_{0-\infty}$. AUC_{0-1} increased

TABLE 4 Pharmacokinetics parameters of all three trials											
	3 µg	10 µg	30 µg	100 µg	300 µg	600 µg	1200 µg	1.25 mg oral solution	200 µg inhaled, with activated charcoal	200 µg inhaled, without activated charcoal	50 µg 1-h intravenous solution
SRD trial											
Subjects	5[#]	6	6	6	6	6	6				
C_{max} pmol·L^{-1}	17.8 (32.9)	50.1 (46.6)	129 (40.1)	462 (53.2)	1090 (26.0)	3130 (30.6)	6500 (73.1)				
t_{max} h	0.083 (0.033–0.083)	0.083 (0.083–0.167)	0.100 (0.083–0.167)	0.134 (0.083–0.183)	0.167 (0.167–0.183)	0.167 (0.083–0.167)	0.183 (0.167–0.250)				
AUC_{0-1} pmol·L^{-1}·h	10.4 (42.4)	28.7 (49.0)	73.7 (43.4)	299 (45.2)	706 (25.9)	1700 (34.5)	3920 (76.4)				
$AUC_{0-\infty}$ pmol·L^{-1}·h	23.8 (88.2)	85.7 (55.3)	208 (37.1)	959 (45.0)	2370 (23.1)	5820 (38.9)	13500 (65.3)				
CL/F mL·min^{-1}	3700 (88.2)	3430 (55.3)	4230 (37.1)	3070 (45.0)	3730 (23.1)	3030 (38.9)	2610 (65.3)				
$t_{1/2}$ h	1.24 (76.2)	2.43 (28.5)	2.70 (35.7)	4.23 (40.2)	6.04 (50.0)	10.2 (23.2)	15.5 (15.2)				
MRD trial											
Subjects		8	8	8	8[¶]	8					
Day 1											
C_{max} pmol·L^{-1}		55.2 (36.1)	158 (27.4)	391 (24.4)	1330 (43.5)	4000 (28.0)					
AUC_{0-12} pmol·L^{-1}·h		82.5 (24.5)	226 (23.2)	734 (22.5)	2430 (35.2)	6320 (21.3)					
t_{max} h		0.083 (0.033–0.167)	0.083 (0.083–0.167)	0.167 (0.083–0.250)	0.167 (0.083–0.250)	0.150 (0.133–0.233)					
$t_{1/2}$ h		2.04 (29.3)	2.44 (16.1)	3.26 (23.2)	7.17 (15.8)	7.43 (19.6)					
$f_{e_{0-12}}$ %		0.721 (37.1)	0.664 (49.5)	0.696 (66.2)	0.766 (65.4)	0.831 (39.8)					
Day 8											
$C_{max,ss}$ pmol·L^{-1}		55.6 (25.7)	143 (38.5)	640 (42.7)	1710 (35.5)	3670 (39.9)					
$t_{max,ss}$ h		0.083 (0.083–0.167)	0.083 (0.083–0.200)	0.125 (0.083–0.250)	0.167 (0.083–0.250)	0.159 (0.117–0.233)					
$AUC_{\tau ss}$ pmol·L^{-1}·h		104 (18.8)	284 (40.0)	1190 (29.4)	3800 (29.3)	7010 (36.4)					
$f_{e_{0-12ss}}$ %		1.06 (25.0)	1.14 (32.8)	1.38 (45.3)	1.36 (54.6)	0.874 (72.9)					
RA_{Cmax}		1.01 (32.9)	0.905 (39.8)	1.64 (32.0)	1.35 (31.7)	0.917 (26.9)					
$RA_{AUC0-12}$		1.26 (22.5)	1.25 (28.1)	1.62 (24.5)	1.60 (28.1)	1.11 (32.0)					

Continued

TABLE 4 Continued										
	3 µg	10 µg	30 µg	100 µg	300 µg	600 µg	1200 µg	1.25 mg oral solution	200 µg inhaled, with activated charcoal	200 µg inhaled, without activated charcoal	50 µg 1-h intravenous solution
Bioavailability											
Subjects								12	12	12	12
C_{max} pmol·L^{-1}								62.5 (49.4)	1910 (25.7)	1800 (31.0)	2040 (17.1)
t_{max} h								0.750 (0.500–1.000)	0.167 (0.083–0.250)	0.167 (0.167–0.250)	0.742 (0.500–0.983)
$AUC_{0-\infty}$ pmol·L^{-1}·h								277 (62.1)	3590 (25.5)	3590 (20.8)	2230 (15.3)
$AUC_{0-\infty}$ pmol·L^{-1}·h											
F %								0.50 (37.8)	40.5 (20.8)	40.2 (16.8)	N/A

Data are presented as n, geometric mean (geometric coefficient of variance) or median (range), unless otherwise stated. SRD: single-rising-dose; C_{max}: maximum measured concentration of BI 1265162 in plasma after single dose; t_{max}: time administered drug excreted from last dosing to the maximum concentration of BI 1265162 in plasma after single dose; AUC_{0-1}: area under the curve of BI 1265162 in plasma from dosing to 1 h post-dosing; $AUC_{0-\infty}$: area under the concentration–time curve of BI 1265162 in plasma over the time interval from 0 extrapolated to infinity; CL/F: apparent clearance; $t_{1/2}$: observed terminal elimination half-life of BI 1265162 in plasma; MRD: multiple-rising-dose; AUC_{0-12}: area under the curve of BI 1265162 in plasma over a 12-h period after single dose; $f_{e_{0-12}}$: fraction of administered drug excreted unchanged in urine over a 12-h period after single dose; $C_{max,ss}$: maximum measured concentration of BI 1265162 in plasma at steady state; $t_{max,ss}$: time administered drug excreted from last dosing to the maximum concentration of BI 1265162 in plasma at steady state; $AUC_{\tau,ss}$: area under the curve of BI 1265162 in plasma over a 12-h period at steady state; $f_{e_{0-12,ss}}$: fraction of administered drug excreted unchanged in urine over a 12-h period at steady state; RA_{Cmax}: accumulation ratio based on $C_{max,ss}$; RA_{AUC}: accumulation ratio based on AUC_{0-12} and $AUC_{\tau,ss}$; F: absolute bioavailability; N/A: not applicable. [#]: n=6 for C_{max} and t_{max}; [¶]: n=7 on day 8.
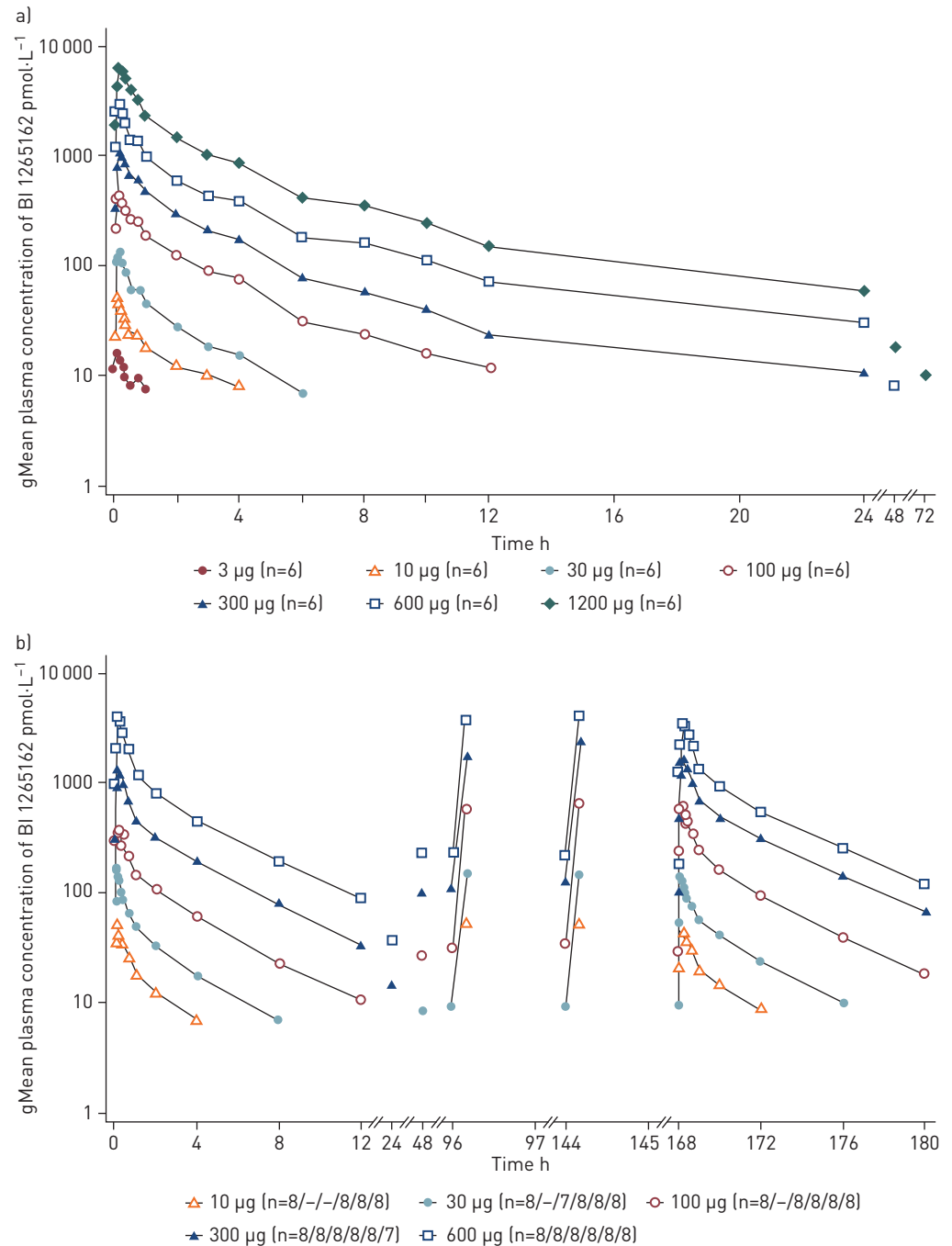
FIGURE 2 Geometric mean plasma concentration–time profiles of BI 1265162 in a) the single-rising-dose trial and b) the multiple-rising-dose trial (doses given twice daily). gMean: geometric mean.

in a dose-proportional manner over 3–1200 μg. Following twice-daily dosing in the MRD, AUC_{0-12} could be estimated more accurately over the 12-h investigational period over the 10–600 μg dose range investigated. Both C_{max} and AUC_{0-12} increased in a dose-proportional manner on day 1 following a single dose and at steady state following twice-daily dosing (10–600 μg). This was confirmed by statistical evaluation as the point estimate for the slope β was close to 1 and the 95% confidence interval included 1 for all PK parameters tested. For doses ⩾100 μg, trough concentrations (12 h post-dose) were approximately ⩽6% of the respective C_{max} values for each subject. Over the entire 6.5-day investigational period of twice-daily dosing, the gMean (geometric coefficient of variance (gCV)) fraction of drug excreted unchanged in urine over a 12-h period was very low for all dose groups (30 μg 0.664% (49.5%) to 100 μg 1.38% (45.3%)).
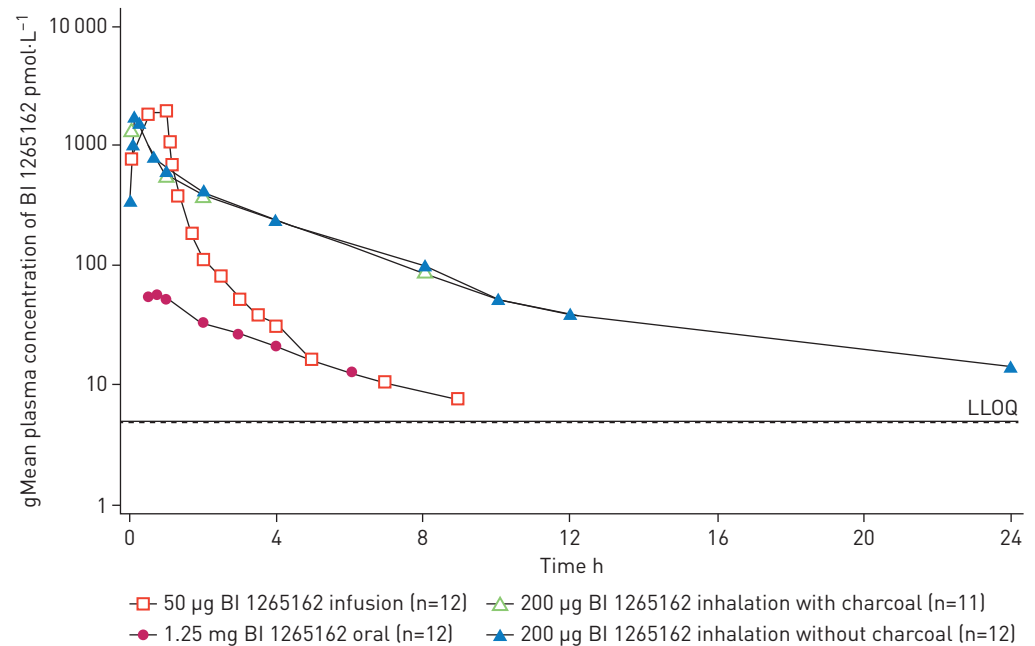
FIGURE 3 Comparison of geometric mean (gMean) plasma concentration–time profiles of BI 1265162 after single administration of BI 1265162 following oral administration (1.25 mg), inhalation with and without charcoal (200 µg) and intravenous infusion (50 µg) (semi-logarithmic scale). LLOQ: lower limit of quantification.

A comparison of gMean plasma concentration–time profiles after a single oral, inhaled (±oral activated charcoal) and *i.v.* administration of BI 1265162 is shown in figure 3. The absolute bioavailability of BI 1265162 following an oral dose of 1.25 mg, estimated as ratio of dose-normalised $AUC_{0-\infty}$ gMeans (gCV) for oral *versus i.v.* administration, was 0.50% (37.8%), whereas the absolute bioavailability (gMean (gCV)) following inhalation of 200 µg BI 1265162 was 40.5% (20.8%) with activated charcoal and 40.2% (16.8%) without. The activated charcoal in the gastrointestinal tract absorbs the drug, thus preventing oral absorption of BI 1265162 into the systemic circulation. This indicates that systemic BI 1265162 exposure represents drug absorbed mainly from the lung.

Discussion

Inhibition of ENaC could restore ASL hydration and enhance mucociliary clearance, and is therefore a promising therapeutic option for patients with CF. In addition, ENaC inhibition may have a synergistic effect with CFTR modulators [9], which restore 10–50% of CFTR function [6–8]. As such, ENaC inhibition could offer a mutation-agnostic approach [9] with therapeutic benefit for all CF patients, irrespective of CFTR genotype or treatment with CFTR modulators. Importantly, in patients who receive CFTR modulator therapy, ENaC inhibition could help to achieve optimal treatment benefit.

We carried out three phase I trials of the inhaled ENaC inhibitor BI 1265162 to assess safety, PK and bioavailability. All tested doses of BI 1265162 were found to be safe and well tolerated in healthy male subjects when administered following single or twice-daily dosing for up to 6.5 days, with adverse events balanced across all groups. All adverse events were of mild or moderate intensity and resolved by trial-end. One subject in the MRD trial experienced hyperkalaemia on day 3, returned to normal during treatment and then increased again, leading to premature discontinuation from the trial drug on day 7 due to an adverse event (hyperkalaemia). The subject recovered within 1 day after treatment discontinuation. All other subjects, except one additional subject (in the bioavailability trial who received three of the four planned doses (the oral dose was missed)), received the trial medication as planned and completed the trial. This subject experienced an SAE (neuropathia vestibularis) leading to hospitalisation. The SAE was not considered drug-related and after recovery, the subject continued in an inhaled-treatment group without reporting any further adverse events. Assessment of palatability and acceptability did not show relevant differences to placebo in the SRD trial, and all but one of the subjects indicated that they would take the medication long term.

Previous clinical development of ENaC inhibitors has been hampered by a number of barriers, including systemic side-effects (chiefly the induction of hyperkalaemia as a result of the high levels of ENaC expression in the cortical collecting duct of the kidney) [20]. It is therefore crucial that any ENaC-blocking

therapy for CF maximises effects in the lung while minimising off-target effects, including ENaC inhibition in the kidneys. In our MRD trial, the subject with hyperkalaemia was asymptomatic, no clinical abnormality was noted in vital signs, especially ECG, and no changes in creatinine clearance were noted. If a blockade of the renal ENaC was involved, a decrease in urine potassium and an increase in urinary sodium and aldosterone could be expected based on the mode of action. However, this was not observed in this subject, whose BI 1265162 PK concentrations and exposures were lower than the highest exposures observed in the higher 600 µg dose group, where no electrolyte changes were observed. No other changes in serum electrolytes, no symptoms or changes in ECG were seen in this subject with a maximum serum potassium at day 6 of 5.91 mmol·L^{-1}. In addition, no serum electrolyte changes were observed following the 1-h infusion of 50 µg BI 1265162 in the bioavailability trial, where gMean C_{max} values were higher than those observed at steady state following inhaled doses of 300 µg twice daily in the MRD trial, whereas gMean $AUC_{0-\infty}$ and AUC_{0-12} values were of similar magnitude following both doses. The reason for this hyperkalaemia remains unclear. Corresponding changes in urine electrolytes are missing and the renal function was stable and normal. It cannot be excluded that individual reasons or susceptibility contributed to the event.

Following inhaled dosing, BI 1265162 was eliminated more slowly from the systemic circulation than after *i.v.* dosing (see the shallower slope after C_{max} in figure 3). This suggests that this phase represents the absorption of drug from the lungs, rather than the elimination of drug from the body, indicating flip-flop kinetics. In this situation, absorption of drug from the lungs is the rate-limiting step. Therefore, the compound's elimination phase becomes dependent on absorption rather than elimination. The oral bioavailability (gMean) of BI 1265162 through the gastrointestinal tract was very low: ∼0.5% compared with the bioavailability following inhalation, which was ∼40% ±oral activated charcoal, administered pre- and post-inhaled dosing. This indicates that systemic BI 1265162 drug concentrations and exposures following inhalation predominately represent drug absorbed from the lung.

BI 1265162 showed dose-proportional PK following both single and twice-daily dosing, with steady state achieved following the second twice daily dose and minimal accumulation. Due to the multi-exponential decline of BI 1265162 observed in the SRD trial, where the true terminal elimination phase appears to start around 12 h post-dose, the selection of AUC_{0-1} instead of $AUC_{0-\infty}$ for evaluation of dose proportionality permitted almost all subjects to be included in the analysis, confirming dose proportionality over the complete dose range tested.

The amount of BI 1265162 excreted unchanged in the urine at steady state was <1.4% of the inhaled dose, showing that renal excretion is not a major elimination route for BI 1265162. Data from rat studies indicate that hepatic elimination is the major route of elimination. A human absorption, metabolism and elimination study is planned to investigate the major routes of elimination.

A potential limitation of this study, as is common with phase I studies in general, is that healthy volunteers were recruited. It is possible that the PK properties of BI 1265162 may be different in healthy volunteers as compared with patients with CF. However, previous studies of a compound related to BI 1265162 demonstrated that PK properties were similar in healthy volunteers and patients with CF (data on file).

In conclusion, results from phase I trials showed that SRDs of BI 1265162 ⩽1200 µg and MRDs of 10 µg, 30 µg, 100 µg, 300 µg and 600 µg were safe and well tolerated in healthy male subjects. Following twice-daily inhalation, BI 1265162 showed dose-linear and time-independent PK within the tested dose ranges. Accumulation was 1.6-fold at maximum. Systemic BI 1265162 drug exposures (given by C_{max}, $AUC_{0-\infty}$ and AUC_{0-1}) represent drug absorbed through the lungs and not through the gastrointestinal tract. BI 1265162 entered phase II clinical development as described in a separate article in *ERJ Open Research* [23].

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