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Phase I Study of the Safety, Tolerability, and Pharmacokinetics of Inhaled Voriconazole in Healthy Volunteers and Subjects With Stable Asthma

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ABSTRACT

The aim of this study was to evaluate safety, tolerability, and pharmacokinetics (PK) of single and multiple doses of a novel inhaled formulation of voriconazole (ZP-059). In the single ascending dose part, 4 cohorts of 6 healthy subjects received one dose of inhaled voriconazole (5–40 mg). In the multiple ascending dose part, 3 cohorts of 6 subjects with mild asthma received voriconazole 10 mg twice daily [BID], 20 mg BID or 40 mg once daily. In the 2-period crossover part, 16 subjects with mild to moderate asthma each received one dose of inhaled voriconazole 20 mg and one dose of oral voriconazole 200 mg. A bioanalytical method was developed and validated to simultaneously determine concentrations of voriconazole and its metabolite N-oxide voriconazole in serum and sputum. Inhaled voriconazole was well tolerated with no treatment emergent adverse events (TEAEs) leading to treatment discontinuation. The PK profile of inhaled voriconazole showed rapid absorption, apparent greater than proportional increase in exposure with increasing dose, a consistent half-life across dosing, and large clearance and volume of distribution. Following repeat administration limited accumulation was observed. Systemic exposure following inhaled voriconazole was much lower than following oral voriconazole. Serum data confirmed that voriconazole was extensively metabolized also when administered by inhalation. Sputum data following inhaled voriconazole were limited but demonstrated increasing exposure with increasing dose. The current study shows the newly developed dry powder inhaled formulation of voriconazole to be safe and well tolerated, providing a possible improved treatment approach for patients affected by allergic bronchopulmonary aspergillosis.

Trial Registration: ClinicalTrials.gov ID: NCT04229303

1 | Introduction

Chronic colonization of the airways with fungi and sensitization to fungal antigens is associated with poor asthma control [1]. Asthmatic patients colonized with *Aspergillus fumigatus* may present with allergic bronchopulmonary aspergillosis (ABPA), characterized by cough, sputum production, wheeze, lung function deterioration, pulmonary infiltrates, and immunological evidence of allergy to *Aspergillus* spp. [2]. The estimated prevalence of ABPA in patients with bronchial asthma, even without

relative standardized diagnostic criteria, is up to 12.9%, regardless of asthma severity [3–5].

ABPA is generally treated with oral corticosteroids and the use of antifungal agents (e.g., azoles) as adjunct therapy has shown improvements in quality of life (QoL), exacerbation rates, and corticosteroid requirements [6–8]. However, long-term treatment with azoles is limited by safety and tolerability issues, including hepatotoxicity and hormone-related adverse effects, such as gynecomastia, alopecia, reduced libido, impotence, oligospermia, hyponatremia, and hypokalemia [9]. Newer oral triazoles with high activity against *Aspergillus* (voriconazole and posaconazole) have been showed promising in the treatment of ABPA [10], with high response rates in patients who discontinued itraconazole due to lack of efficacy or side effects [11].

Voriconazole has similar in vitro minimum inhibitory concentrations against *Aspergillus fumigatus* as itraconazole and posaconazole but has the potential to provide an improved safety and pharmacokinetic (PK) profile, with good penetration into lung tissue and epithelial lining fluid [12]. However, despite promising efficacy in ABPA patients and the improved PK profile, prolonged use of systemic voriconazole is still limited by frequent side effects such as peripheral oedema, headache, respiratory distress, nausea, diarrhea and abnormal liver function test results and treatment discontinuations.

Administration of voriconazole via inhalation could offer an effective antifungal therapeutic option for ABPA with lower systemic exposure, less side effects compared to systemic administration and lower potential for drug–drug interactions due to cytochrome P450 enzyme inhibition [13]. An inhaled formulation of voriconazole has been engineered based on an innovative technology (E_{dry}) to provide dry powder particles of optimal size, aerodynamic performance and de-agglomeration characteristics [14] endowed with more efficient lung deposition, low systemic exposure and thus reduced side effects.

The aim of the current Phase I study was to evaluate the safety, tolerability, and PK of single and multiple doses of the inhaled formulation of voriconazole in healthy subjects and subjects with stable asthma, with sputum sampling to evaluate lung exposure, and a comparison of PK following inhaled and oral administration in a crossover part.

2 | Methods

2.1 | Study Subjects

The study consisted of three parts, single ascending dose (SAD), multiple ascending dose (MAD), and a 2-period crossover. In the SAD part, eligible subjects were healthy adult male or females with no history of severe cough or bronchospasm after inhaling an inhalation product. In the MAD part, eligible subjects were adult male or females with mild stable asthma diagnosed at least 3 months prior to screening and receiving low to medium doses of inhaled corticosteroids with or without short acting beta-agonists. In the 2-period crossover part, eligible subjects were adult male and females with mild to moderate stable asthma and receiving low to medium doses of inhaled corticosteroids. All subjects with asthma were required to have no current diagnosis of any other chronic airway disease and no history of any life-threatening asthma episodes.

Exclusion criteria included a smoking history of >5 pack years at screening, or a history of allergy or hypersensitivity to voriconazole, its excipients or to other antifungal azoles. Chinese or Japanese subjects were not enrolled into the study.

All subjects provided written informed consent to participate in the study. The protocol and informed consent form were approved by an Independent Ethics Committee. The study was performed in accordance with the Declaration of Helsinki and Council for International Organizations of Medical Sciences International Ethical Guidelines, and the requirements of the International Council for Harmonization (ICH) and Good Clinical Practice (GCP) guidelines. The study was registered in the ClinicalTrials.gov registry (ClinicalTrials.gov ID: NCT04229303).

2.2 | Study Design

This Phase I study was of open-label, single center design and was performed at a Phase I Unit in the UK. Subjects were screened for eligibility to participate in the study within 28 days before dosing (Day 1) and were admitted to the study site on the evening of Day −1.

The study integrated a single ascending dose (SAD) part in healthy subjects (Part 1), a multiple ascending dose (MAD) part in subjects with mild stable asthma (Part 2), and a 2-period crossover part comparing single doses of inhaled and oral voriconazole in subjects with mild to moderate stable asthma (Part 3).

In Part 1 (SAD), four cohorts of six healthy subjects received single doses of inhaled voriconazole on the morning of Day 1. The starting dose was 5 mg, with subsequent dose escalations to 10 mg, 20 mg, and 40 mg. Safety data was reviewed prior to escalation to the next dose level by a Safety Advisory Committee (SAC) which comprised at least the principal investigator (PI) or delegate and the medical monitor of Zambon S.p.A.

In Part 2 (MAD), three cohorts of six subjects with mild asthma received repeat doses of inhaled voriconazole each day from Day 1 to Day 10. The starting dose was 10 mg twice daily (BID), with subsequent doses of 20 mg BID and 40 mg once daily (OD). Part 2 could only start once the SAC confirmed it could proceed based on review of safety data from Part 1.

Part 3 (2-period crossover) was a randomized, crossover study in 16 subjects with mild to moderate asthma. Subjects received a single inhaled dose of voriconazole 20 mg and a single dose of oral voriconazole 200 mg on the morning of Day 1 of the respective treatment period, with a washout period between treatments of at least 96 h. Subjects were randomly allocated to the sequence of treatment periods.

2.3 | Study Medication

Voriconazole for inhalation (ZP-059) was provided by Zambon S.p.A. as clear, colorless, hard capsules (nominal dose strength 5 mg per capsule) for administration via a breath actuated dry powder inhaler (RS01 monodose inhaler, Plastiape, S.p.A., Italy). Commercially available voriconazole film-coated tablets for oral administration (Vfend) were purchased for Part 3.

2.4 | Samples

For serum PK evaluation, venous blood was withdrawn via an indwelling catheter or by venipuncture at pre-specified timepoints (Part 1: pre-dose, 1.5, 2, 3, 4, 12 h post-dose; Part 2: pre-dose, 1.5, 2, 3, 4, 12, 24 h post-dose on Day 1 and Day 10, prior to 0-h dosing on Day 3–9; Part 3: pre-dose, 0.25, 0.75, 1.5, 2, 3, 4, 6, 8, 12, 16, 24 and 48 h post-dose). More frequent PK sampling was scheduled for Part 3 to collect seamless and complete information as a comparator for future clinical development studies of inhaled voriconazole in ABPA.

In a limited subset of subjects able to provide a sputum sample, sputum PK was evaluated at prespecified timepoints (Part 2: Day 7 pre-dose, 3 and 6 h post-dose; Part 3: first 3 days of each treatment period at 2, 6, 24 and 48 h post-dose). Sputum induction was performed using saline inhalation in accordance with European Respiratory Society guidelines [15] and study site standard procedure. Serum and sputum samples were sent to the analytical laboratory (Alderley Analytical Ltd) for simultaneous determination of voriconazole and its main metabolite N-oxide concentrations, by a validated liquid chromatography and tandem mass spectrometry method.

2.5 | Bioanalytical Method

The bioanalytical method validation was performed according to the EMA and FDA guidelines (Guideline on Bioanalytical Method Validation, February 2012 and The Guidance for Industry on Bioanalytical Method Validation, May 2018, respectively). Full validation was conducted for the determination of voriconazole and N-oxide voriconazole concentrations in human serum (range 0.5–500 ng/mL) and in human sputum (range 0.2–200 ng/mL). Validation included the assessment of linear range and response factor, intra- and inter-run precision and accuracy, sensitivity, dilution integrity, recovery, selectivity, matrix effect, carryover and stability.

2.6 | Analysis

Safety and tolerability endpoints included the frequency of treatment-related adverse events (TEAEs), serious adverse events (SAEs), adverse events of special interest (AESIs: moderate or severe dyspnea, wheeze, cough, or bronchospasm; moderate or severe visual impairment/ hallucination; anaphylaxis and severe allergic reaction; hepatotoxicity $\geq 3 \times$ ULN for AST, ALT, and bilirubin), physical examination and changes in vital signs, clinical laboratory parameters (clinical chemistry, hematology, urinalysis), 12-lead ECG, pulse oximetry, and spirometry assessments (forced expiratory volume in 1 s [FEV_1], forced vital capacity [FVC], peak expiratory flow rate and FEV_1/FVC ratio).

Serum PK parameters were calculated for voriconazole and N-oxide voriconazole after single (Day 1 of Parts 1, 2 and 3) and multiple dosing (Part 2 Day 10 used for steady state). An exploratory endpoint for Parts 2 and 3 was voriconazole sputum concentrations depending on data availability.

No formal sample size calculation was performed and there was no formal hypothesis testing in the study. The sample size for each part was chosen to minimize investigational medicinal product exposure while providing an adequate number of subjects for assessment of safety and mean PK parameters.

Analysis was performed using descriptive statistics only. Serum and sputum concentration data were analyzed using PK non-compartmental analysis (Phoenix WinNonlin version 8.2.0.04383).

3 | Results

3.1 | Study Subjects

A total of 58 subjects were enrolled (37 males and 21 females). There were no important findings with respect to demographics, baseline characteristics, medical history or concomitant medication.

Part 1 enrolled 24 healthy subjects (11 female and 13 male) with a mean age of 42.5 years. All subjects were White except for one Black subject in cohort 2 (10 mg dose). 19 subjects had never smoked and 5 were former smokers. Demographics and baseline characteristics were well balanced across cohorts.

Part 2 enrolled 18 subjects with mild asthma (7 female, 11 male) with a mean age of 36.9 years. All subjects were White except for 1 Black subject in Cohort 1 (10 mg BID) and one mixed Asian/White subject in Cohort 2 (20 mg BID). Sixteen subjects had never smoked and 2 were former smokers. Subject characteristics were well balanced across cohorts (Table 1a).

Part 3 enrolled 16 subjects with mild to moderate asthma (3 female, 13 male) with a mean age of 29.9 years. All subjects were white except for one Asian subject and one British Bengali subject. Fourteen patients had a diagnosis of mild asthma while 2 subjects were diagnosed with moderate asthma. Fourteen subjects had never smoked and 2 were former smokers (Table 1b).

All subjects received the planned study medication and completed the study, with no major protocol violations.

3.2 | Bioanalytical Method Validation

The HPLC-MS/MS analytical method for the determination of voriconazole and N-oxide voriconazole concentrations in human serum and sputum samples met the acceptance criteria and was therefore considered suitable for the analysis of study samples. In particular, the analysis of serum quality control samples (QCs) demonstrated an intra-run precision $\leq 4.4\%$ (voriconazole) and $\leq 9.9\%$ (N-oxide voriconazole), and an

TABLE 1A | Subject characteristics (Part 2, multiple ascending dose).

	10 mg	20 mg	40 mg	Overall
	BID ($n=6$)	BID ($n=6$)	BID ($n=6$)	($n=18$)
Age (y)				
Mean	38.3	39	33.5	36.9
Range (min, max)	31, 45	32, 48	20, 50	20, 50
Sex				
Male	4	4	3	11
Female	2	2	3	7
Race				
Black or African American	1	0	0	1
White	5	5	6	16
Other	0	1	0	1
Body mass index at screening ($kg*m^{-2}$)				
Mean	27.4	26.9	23.98	26.09
Range (min, max)	24.9, 31.8	23.2, 32.0	20.4, 26.3	20.4, 32.0
Time since asthma diagnosis (y)				
Mean	30.48	28.94	18.62	26.01
Range (min, max)	22.4, 42.4	11.4, 47.5	3.9, 40.5	3.9, 47.5
Concomitant medications				
Inhaled Corticosteroids	4	1	3	8
Short-acting beta agonists	6	6	6	18
Long-acting beta agonists	0	0	0	0
FEV_1 liters (mean; SD)				
Baseline	3.41; 0.94	3.41; 0.40	3.56; 0.37	n.a.

inter-run precision ≤ 3.2% (voriconazole) and ≤ 10.0% (N-oxide voriconazole). The intra-run accuracy ranged 94.7%–105% and 88.0%–107% for voriconazole and N-oxide voriconazole, respectively and the inter-run accuracy was ≤ 103% for both analytes.

The analysis of sputum QCs demonstrated an intra-run precision ≤ 7.7% (voriconazole) and ≤ 9.6% (N-oxide voriconazole), and an inter-run precision ≤ 5.4% (voriconazole) and ≤ 6.8% (N-oxide voriconazole). The intra-run accuracy ranged 99.3%–110% and 98.0%–113% for voriconazole and N-oxide voriconazole, respectively and the inter-run accuracy was ≤ 107% for voriconazole and ≤ 110% for N-oxide voriconazole.

3.3 | Voriconazole Pharmacokinetics

3.3.1 | Single Dose

Mean serum concentrations of voriconazole over time after single doses of 5, 10, 20, and 40 mg inhaled formulation (Part 1) are shown in Figure 1a.

Voriconazole appeared to be rapidly absorbed into the systemic circulation when administered by inhalation. The peak plasma concentration occurred at 1.5 h after drug administration at all doses (Table 2). There was an apparent greater than dose proportional increase in voriconazole $AUC_{0\text{-}t}$ and C_{max} across the dose range. For example, for an eightfold increase in dose (5–40 mg) there was an approximate 14-fold increase in voriconazole AUC. This was confirmed by an analysis of dose proportionality with slope (β) estimated from the power model fitted to $AUC_{0\text{-}t}$ = 1.21 (90% CI: 1.050, 1.362), indicating a greater than dose proportional increase in exposure across the dose range. However, the observed deviation from linearity may be due to a relatively poor description of serum concentration versus time profiles at the lowest dose (5 mg) that precludes an accurate calculation of AUC values. As a further support to this, the clearance values remained consistently stable throughout the dose range.

Mean $t_{1/2}$ was approximately 3 h and mean apparent volume of distribution was high, suggesting a high distribution into extravascular compartments. Intersubject variability across the respective PK parameters (CV% of the arithmetic mean) was relatively low (mainly ≤ 30%).

TABLE 1B | Subject characteristics (Part 3, crossover).

	Inhaled/oral ($n=8$)	Oral/inhaled ($n=8$)	Overall ($n=16$)
Age (years)			
Mean	33.8	26.0	29.9
Range (min, max)	19, 50	20, 33	19, 50
Sex			
Male	7	6	13
Female	1	2	3
Race			
Asian	1	1	2
White	6	7	13
Other	1	0	1
Body mass index at screening ($kg*m^{-2}$)			
Mean	24.83	24.96	24.89
Range (min, max)	19.2, 31.5	22.2, 26.6	19.2, 31.5
Time since asthma diagnosis (years)			
Mean	23.57	18.61	21.09
Range (min, max)	1.5, 45.5	5.5, 32.5	1.5, 45.5
Asthma severity (n)			
Mild	8	6	14
Moderate	0	2	2
Concomitant medications			
Inhaled Corticosteroids	8	8	16
Short-acting beta agonists	8	8	16
Long-acting beta agonists	0	0	0

FEV_1 liters (mean; SD)	**Inhaled voriconazole ($n=16$)**	**Oral voriconazole ($n=16$)**
Baseline	3.68; 0.40	3.72; 0.41

3.3.2 | Multiple Dosing

PK parameters and concentrations over time profiles on Day 1 and Day 10 after multiple doses of inhaled voriconazole are shown in Table 3 and Figure 1b,c, respectively.

On both Days 1 and 10, median T_{max} was 1.5 h over the whole dose range. On Day 1 mean serum exposure (C_{max} and $AUC_{0\text{-}t}$) increase was dose proportional between the 10 mg and 20 mg BID doses but was slightly more than dose proportional between 20 mg BID and 40 mg OD doses. The PK parameters obtained on Day 1 confirmed the PK parameters obtained during Part 1 of the study.

Conversely on Day 10 exposure parameters were greater than dose proportional between 10 mg and 20 mg BID but linearity was observed between the 20 mg BID and 40 mg OD doses. Geometric mean values for $C_{ss,av}$ on Day 10 ranged from 6.91 ng/mL with 10 mg BID to 35.64 ng/mL with 40 mg OD. For all doses there was very limited accumulation of drug following multiple dosing (mean accumulation ratio [Rac]: 1.19, 1.55, and 1.30 for 10 mg, 20 mg, and 40 mg groups, respectively).

Trough serum concentrations (always <13 ng/mL) collected throughout the dosing period suggested that steady state for voriconazole was reached between Days 2 and 4 following repeat administration. Fluctuation in serum concentrations was consistent across dosing regimens: the geometric means were 281% and 284.9% for 10 and 20 mg BID, respectively, with greater fluctuation [350.8%] for the 40 mg OD regimen.

3.3.3 | Inhaled vs. Oral Dosing

Figure 2 shows mean serum concentrations of voriconazole over time after a single inhaled dose (20 mg) and an oral dose (200 mg), with PK parameters of voriconazole and N-oxide voriconazole for the 2 routes of administration shown in Table 4.

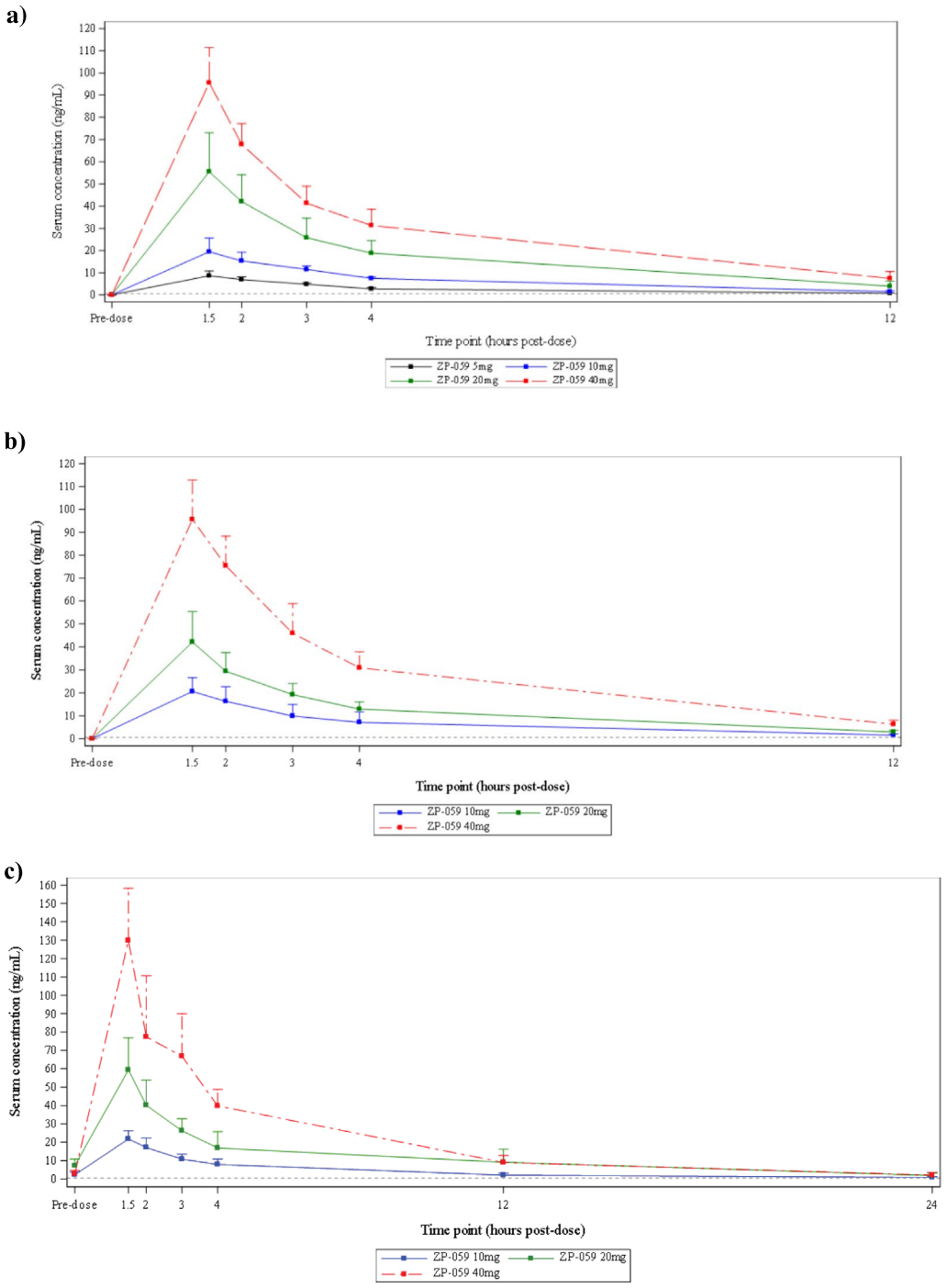

FIGURE 1 | (a) Serum Concentration of Voriconazole Over Time after Single Inhaled Doses (Mean [+SD], Part 1, Day 1). (b) Serum Concentration of Voriconazole Over Time after Single Inhaled Doses (Mean [+SD], Part 2, Day 1). (c) Serum Concentration of Voriconazole Over Time after Multiple Inhaled Doses (Mean [+SD], Part 2, Day 10).

Systemic absorption was faster following inhaled voriconazole than after oral administration (median T_{max}: 0.25 h and 1.5 h, respectively). Serum concentrations at 2 h post dose were 37.1 and 635.6 ng/mL following inhalation and oral administration, respectively. At 6 h post dose these concentrations decreased to 10.5 and 202.1 ng/mL, respectively. As expected, the rate and extent (C_{max} and AUC) of systemic exposure of voriconazole for the inhaled formulation was much lower (≈10%) than that of the oral formulation (Vfend). Clearance, volume of distribution and half-life were comparable between the two different formulations.

3.3.4 | Sputum Samples

Sputum samples were obtained for 27 subjects (12 in Part 2 and 15 in Part 3). The number of adequate samples and with quantifiable voriconazole concentration was limited. However, in Part

TABLE 2 | Serum pharmacokinetic parameters after single doses of inhaled voriconazole (geometric mean [SD], Part 1).

Parameter	5 mg ($n=6$)	10 mg ($n=6$)	20 mg ($n=6$)	40 mg ($n=6$)
Voriconazole				
$AUC_{0\text{-}t}$, h*ng/mL	24.36 (1.45)[a]	73.89 (1.16)	187.76 (1.35)	328.37 (1.19)
$AUC_{0\text{-}inf}$, h*ng/mL	40.00 (1.00)	78.94 (1.18)	203.96 (1.44)	377.19 (1.20)
C_{max}, ng/mL	8.44 (1.27)	18.52 (1.39)	53.37 (1.32)	94.33 (1.18)
T_{max}, h[b]	1.50	1.50	1.50	1.50
Kel, 1/h	0.30 (1.52)	0.23 (1.09)	0.22 (1.17)	0.19 (1.22)
$t_{1/2}$, h	2.67 (1.42)[c]	3.07 (1.09)	3.20 (1.17)	3.63 (1.22)
CL/F L/h	131.2 (1.04)	124.6 (1.17)	97.7 (1.39)	108.7 (1.22)
Vz/F, L	614.3 (1.09)	553.1 (1.14)	450.9 (1.25)	569.6 (1.20)
N-oxide voriconazole				
$AUC_{0\text{-}t}$, h*ng/mL	298.15 (1.11)	573.55 (1.26)	804.24 (1.11)	1835.81 (1.09)
$AUC_{0\text{-}inf}$, h*ng/mL	337.40 (1.12)	652.99 (1.32)	865.80 (1.07)	1977.21 (1.11)
C_{max}, ng/mL	57.70 (1.12)	103.16 (1.32)	145.79 (1.30)	286.63 (1.15)
T_{max}, h[b]	1.50	1.50	1.75	1.50
Kel, 1/h	0.19 (1.08)	0.19 (1.21)	0.20 (1.27)	0.16 (1.26)
$t_{1/2}$, h	3.60 (1.08)	3.59 (1.21)	3.38 (1.27)	4.43 (1.26)

Abbreviations: $AUC_{0\text{-}inf}$ = area under the serum concentration time curve from time 0 to infinity, $AUC_{0\text{-}t}$ = area under the serum concentration time curve from time 0 to time t, CL/F = apparent clearance, C_{max} = maximum concentration, Kel = elimination rate constant, SD = standard deviation; $t_{1/2}$ = elimination half-life; T_{max} = time to maximum concentration; Vz/F = apparent volume of distribution.
[a]t = 4 h for three out six subjects.
[b]Median.
[c]$n=4$, one subject with $t_{last}=4$ h.

2 the results show increasing concentrations of voriconazole with increasing dose between 10 and 20 mg BID and similar pre-dose concentrations in patients receiving the same dose (Table 5a). Very few samples were collected in patients treated with 40 mg OD, hampering any evaluation. In Part 3, the concentrations of voriconazole in sputum samples were lower after inhaled vs. oral voriconazole approximately in keeping with the 10-fold lower inhaled dose. High intersubject variability was observed, particularly following inhalation. At 48 h post-dose voriconazole concentrations were below the limit of quantification both after inhaled and oral voriconazole, with only some exceptions (Table 5b).

3.3.5 | N-Oxide Voriconazole Concentrations

The main metabolite (N-oxide voriconazole) concentrations were determined both in serum and in sputum samples following single and repeated administrations of voriconazole by inhalation.

After single dosing, at doses of 5 and 10 mg, the N-oxide voriconazole C_{max} and AUC values were approximately 6- and 10-fold higher than voriconazole C_{max} and AUC values, respectively. These metabolic ratios were lower at the highest doses of 20 and 40 mg, being approximately 3 and 6 for C_{max} and AUC, respectively (Table 2). Following multiple dosing, the N-oxide voriconazole/voriconazole ratios for C_{max} and AUC on Days 1 and 10 were reasonably constant across the dose range. On Day 10 the metabolite ratios were only slightly higher than those observed on Day 1. In Part 3, the N-oxide voriconazole/voriconazole ratios for C_{max} were similar following inhalation and oral administration, whereas the N-oxide voriconazole/voriconazole ratio for AUC was higher following oral than following inhalation (Table 4).

N-oxide voriconazole concentrations determined in sputum samples were generally higher than sputum voriconazole concentrations both in Part 2 and in Part 3 (Table 5a,b).

3.4 | Safety and Tolerability

Overall voriconazole was well tolerated with no serious TEAEs, severe TEAEs or TEAEs leading to discontinuation of treatment. All TEAEs reported during the study were mild to moderate.

Part 1: A total of 7 (29.2%) subjects reported TEAEs (Table 6a). None were considered to be treatment-related. There was no pattern of type of TEAEs reported, with the only TEAE reported by more than one subject being headache (2 subjects in the 40 mg group).

Part 2: A total of 13 (72.2%) subjects reported TEAEs (Table 6b). Of these, 5 were possibly treatment-related (10 mg: 3 subjects with dysgeusia [distortion of taste] and 1 subject with wheezing,

TABLE 3 | Serum pharmacokinetic parameters after multiple doses of inhaled voriconazole (geometric mean [SD], Part 2, Day 1 and 10).

Parameter	10 mg BID ($n=6$)	20 mg BID ($n=6$)	40 mg OD ($n=6$)
DAY 1			
AUC_{0-t}, h*ng/mL	69.65 (1.39)	139.29 (1.21)	329.28 (1.19)
AUC_{0-inf}, h*ng/mL	78.20 (1.40)	155.72 (1.16)	358.13 (1.20)
C_{max}, ng/mL	19.87 (1.29)	40.42 (1.33)	96.77 (1.16)
T_{max}, h[a]	1.50	1.50	1.50
Kel, 1/h	0.19 (1.35)	0.20 (1.25)	0.21 (1.08)
$t_{1/2}$, h	3.60 (1.36)	3.53 (1.25)	3.24 (1.08)
DAY 10			
AUC_{0-t}, h*ng/mL	91.00 (1.36)	256.04 (1.41)	480.22 (1.27)
AUC_{0-inf}, h*ng/mL	97.78 (1.35)	258.66 (1.44)	493.04 (1.29)
C_{max}, ng/mL	21.38 (1.21)	57.11 (1.33)	127.54 (1.20)
T_{max}, h[a]	1.50	1.50	1.50
Kel, 1/h	0.16 (1.41)	0.13 (1.29)	0.15 (1.15)
$t_{1/2}$, h	4.40 (1.41)	5.15 (1.29)	4.48 (1.15)
CL/F, L/h	120.9 (1.29)	93.1 (1.32)	93.5 (1.25)
Vz/F, L	767.1 (1.47)	714.3 (1.25)	604.9 (1.13)
AUC_{tau}, h*ng/mL	82.82 (1.30)	215.56 (1.32)	427.57 (1.25)
$C_{ss, av}$, ng/mL	6.91 (1.30)	17.95 (1.32)	35.64 (1.25)
Fluctuations %	281.0 (1.15)	284.9 (1.24)	350.8 (1.10)
Rac	1.19 (1.17)	1.55 (1.29)	1.30 (1.27)

Abbreviations: AUC_{0-inf} = area under the serum concentration time curve from time 0 to infinity, AUC_{0-t} = area under the serum concentration time curve from time 0 to time t, AUC_{tau} = area under the serum concentration time curve for the dosing interval, BID = twice daily, CL/F = apparent clearance, C_{max} = maximum concentration, $C_{ss, av}$ = average drug concentration at steady state, Kel = elimination rate constant, OD = once daily, Rac = accumulation ratio, SD = standard deviation, $t_{1/2}$ = elimination half-life, T_{max} = time to maximum concentration, Vz/F = apparent volume of distribution.
[a]Median.

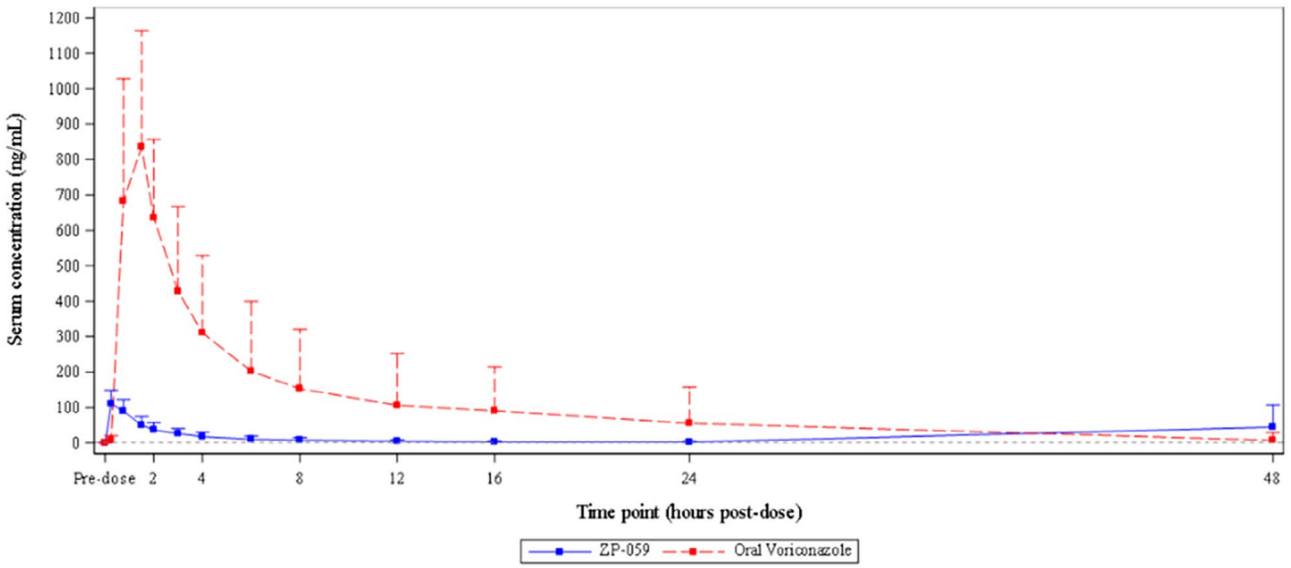

FIGURE 2 | Serum Concentration of Voriconazole Over Time after a Single Inhaled Dose (20 mg) and a Single Oral Dose (200 mg) (Mean [+SD], Part 3, Day 1).

TABLE 4 | Serum pharmacokinetic parameters after a single inhaled dose (20 mg) and a single oral dose (200 mg) of voriconazole (geometric mean [SD], Part 3).

Parameter	Inhaled voriconazole 20 mg ($n=16$)	Oral voriconazole 200 mg ($n=16$)
Voriconazole		
$AUC_{0\text{-}t}$, h*ng/mL	290.02 (1.87)	3726.68 (1.91)
$AUC_{0\text{-}inf}$, h*ng/mL	269.61 (1.56)	3800.41 (1.92)
C_{max}, ng/mL	108.08 (1.42)	863.22 (1.44)
T_{max}, h[a]	0.25	1.50
Kel, 1/h	0.14 (1.26)	0.10 (1.31)
$t_{1/2}$, h	5.13 (1.27)	6.93 (1.32)
CL/F, L/h	74.2 (1.57)	52.4 (1.93)
Vz/F, L	549.0 (1.46)	526.0 (1.69)
N-oxide voriconazole		
$AUC_{0\text{-}t}$, h*ng/mL	1128.69 (1.27)	21504.52 (1.20)
$AUC_{0\text{-}inf}$, h*ng/mL	1154.35 (1.26)	21788.46 (1.20)
C_{max}, ng/mL	151.43 (1.25)	1589.05 (1.25)
T_{max}, h[a]	0.25	1.50
Kel, 1/h	0.15 (1.19)	0.11 (1.34)
$t_{1/2}$, h	4.68 (1.19)	6.42 (1.35)
MR $AUC_{0\text{-}t}$	3.89 (1.92)	5.77 (1.81)
MR $AUC_{0\text{-}inf}$	4.38 (1.48)	5.74 (1.79)
MR C_{max}	1.40 (1.44)	1.84 (1.54)

Abbreviations: $AUC_{0\text{-}inf}$ = area under the serum concentration time curve from time 0 to infinity, $AUC_{0\text{-}t}$ = area under the serum concentration time curve from time 0 to time t, CL/F = apparent clearance, C_{max} = maximum concentration, Kel = elimination rate constant, SD = standard deviation, $t_{1/2}$ = elimination half-life, T_{max} = time to maximum concentration, Vz/F = apparent volume of distribution, MR = metabolic ratio.
[a]Median.

chest discomfort and cough; 40 mg: 1 subject with throat irritation and wheezing). Other than dysgeusia the only TEAEs reported by more than one subject were abdominal distension (2 in the 20 mg group) and headache (1 in the 10 mg group, 1 in the 20 mg group, and 2 in the 40 mg group).

Part 3: A total of 9 (56.3%) subjects reported a TEAE after inhaled voriconazole. Of these 7 were considered at least possibly treatment-related (all mild chest discomfort temporarily related to inhalation) (Table 6c). After oral voriconazole 7 (43.8%) subjects reported TEAEs, none of which were treatment-related. Other than mild chest discomfort (7 subjects on inhaled, 1 subject on oral) the only TEAE reported in more than one subject was headache (4 subjects on inhaled, 3 on oral).

No AESIs were reported (moderate or severe dyspnea, wheezing, cough or bronchospasm, visual impairment/hallucination, anaphylaxis and severe allergic reaction, or hepatotoxicity ≥ 3 × ULN for AST, ALT, and bilirubin).

No notable or clinically relevant changes were observed for physical examination, vital signs, clinical laboratory values, ECG and pulse oximetry. No decrease in lung function was observed following administration of inhaled voriconazole.

4 | Discussion

This is the first report on the safety, tolerability, and PK of a novel inhaled formulation of voriconazole (ZP-059), with PK characterization of its main metabolite, N-oxide voriconazole, after inhalation.

The PK of voriconazole following IV or oral administration is well established, characterized by rapid absorption, non-linear kinetics due to saturation of hepatic metabolism, greater than proportional increase in exposure with increasing dose, dose-dependent terminal half-life, and large volume of distribution [16, 17]. Rapid absorption and large volume of distribution were observed also in the current study following inhaled voriconazole. In particular, the time to maximum concentration observed at 15 min in the crossover part of this study demonstrates that voriconazole is absorbed into the systemic circulation from the lung, as absorption from the gut of a swallowed portion of the inhaled dose would have occurred later. Consistent with this, T_{max} following oral voriconazole is 1.5 h. The half-life across doses is constant and the pharmacokinetics following multiple dosing of inhaled voriconazole are characterized by very limited accumulation, in contrast to that observed following systemic administration.

The intersubject variability in terms of serum exposure after inhaled voriconazole was relatively low compared to the very high interindividual variability usually observed following systemic administrations of voriconazole. A direct comparison with the oral administration (Part 3) shows that the systemic exposure following inhalation was lower than following oral administration, consistent with a 10-fold lower administered dose and that the concentrations of voriconazole determined in sputum samples were higher following the oral dose (200 mg) than following the inhaled dose (20 mg). However, at 2 h post-dose the voriconazole sputum/serum ratio is higher after inhalation than after oral administration and the ratio between N-oxide and voriconazole concentrations in sputum samples is much lower when voriconazole is administered by inhalation. More data are needed to understand if these ratios may support the inhalation route as a more favorable route of administration, even because at 6 h the sputum/serum and the N-oxide/voriconazole ratio post inhaled dose are comparable to sputum/serum and N-oxide/voriconazole ratio post oral dose.

PK parameters and serum concentration of voriconazole over time profiles obtained on Day 1 in Part 1 and on Day 1 in Part 2 of the present study are similar. As Part 1 enrolled healthy subjects and Part 2 enrolled subjects with mild asthma, this suggests that the pharmacokinetics of inhaled voriconazole

TABLE 5A | Induced sputum concentrations of voriconazole and N-oxide voriconazole (Part 2, Day 7, single concentration values).

	Voriconazole (ng/mL) $N=12$						N-Oxide voriconazole (ng/mL) $N=12$					
10 mg BID ($N=6$)												
pre dose	0.3	0.7	0.3	blq	0.3	0.2	2.9	6.5	3.4	2.8	3.5	1.5
3 h post-dose	1.0	NR	0.8	1.1	NR	2.1	15.3	NR	11.2	11.9	NR	10.6
6 h post-dose	NR	NR	0.3	0.4	NR	0.5	NR	NR	5.9	7.6	NR	5.2
20 mg BID (N*$=4$)												
pre dose	0.7	0.8	0.8				9.2	10.3	12.0			
3 h post-dose	2.4	2.6	NR				30.2	33.5	NR			
6 h post-dose	1.2	1.3	1.2				18.3	19.7	22.7			
40 mg ($N=2$)												
pre dose	0.3	0.2					5.5	1.9				
3 h post-dose	158.0	NR					66.0	NR				
6 h post-dose	3.8	NR					57.2	NR				

Abbreviations: blq = below limit of quantification (0.2 ng/mL), N = number of subjects who receive the treatment and able to produce a sputum sample, NR = no sample taken.
*One patient was excluded due to bioanalytical issues.

TABLE 5B | Descriptive statistics of induced sputum concentrations of voriconazole and N-oxide voriconazole after inhaled and oral voriconazole (Part 3).

		Voriconazole (ng/mL) $N=15$		N-oxide voriconazole (ng/mL) $N=15$	
		Inhaled voriconazole	**Oral voriconazole**	**Inhaled voriconazole**	**Oral voriconazole**
2 h post-dose	n (NR)	11 (4)	10 (5)	11 (4)	10 (5)
	Mean	38.3	50.5	28.3	284.2
	SD	114.0	28.9	6.2	126.7
	CV%	297.7	57.2	21.8	44.6
6 h post-dose	n (NR)	12 (3)	12 (3)	12 (3)	12 (3)
	Mean	2	29.1	16.4	201.6
	SD	3.0	24.6	5.6	61.3
	CV%	149.5	84.5	34.4	30.4
24 h post-dose	n (NR)	2 (1)	12 (3)	14 (1)	12 (3)
	Mean	*0.4; *0.5	3.3	1.0	51.5
	SD		1.5	0.4	20.9
	CV%		47.1	43.9	40.6
48 h post-dose	n (NR)	0 (2)	4 (2)	2 (2)	12 (2)
	Mean		2.6	*0.3; *1.1	4.6
	SD		4.4		6.8
	CV%		167.0		147.6

Abbreviations: N = number of subjects who receive the treatment, n = number of subjects with quantifiable concentrations; NR = no sample taken.
*Single concentration values.

might not be affected by the conditions of this target population. The differences observed between the PK parameters and PK profiles obtained in Part 1 (healthy subjects) and in Part 3 (mild to moderate stable asthma subjects) following inhaled voriconazole are not of concerns in that respect, being in line with the different sampling time points. Interestingly, when voriconazole concentrations determined at the same sampling time points collected in Part 1 and Part 3 (i.e., at 1.5, 2,

TABLE 6 | Summary of treatment-emergent adverse events.

(a) PART 1				
Inhaled Voriconazole (Single Dose)				
n **(%)**	**5 mg (*n* = 6)**	**10 mg (*n* = 6)**	**20 mg (*n* = 6)**	**40 mg (*n* = 6)**
TEAE	0	3 (50.0)	2 (33.3)	2 (33.3)
Intensity				
Mild	0	2 (33.3)	2 (33.3)	1 (16.7)
Moderate	0	1 (16.7)	0	1 (16.7)
Treatment-related	0	0	0	0

(b) PART 2			
Inhaled voriconazole (multiple dose)			
n **(%)**	**10 mg BID (*n* = 6)**	**20 mg BID (*n* = 6)**	**40 mg OD (*n* = 6)**
TEAE	5 (83.3)	4 (66.7)	4 (66.7)
Intensity			
Mild	3 (50.0)	3 (50.0)	2 (33.3)
Moderate	2 (33.3)	1 (16.7)	2 (33.3)
Treatment-related	4 (66.7)	0	1 (16.7)

(c) PART 3		
	Inhaled Voriconazole	**Oral Voriconazole**
n **(%)**	**20 mg (*n* = 16)**	**200 mg (*n* = 16)**
TEAE	9 (56.3)	7 (43.8)
Intensity		
Mild	8 (50.0)	5 (31.3)
Moderate	1 (6.3)	2 (12.5)
Treatment-related	7 (43.8)	0

3, 4 and 12 h) were compared, a good overlapping is observed, strengthening the hypothesis that these pathological conditions have a limited impact on the pharmacokinetics of the inhaled voriconazole. Anyhow, it cannot be excluded that more severe conditions may affect the PK profile of the compound administered by inhalation. Very recently published data reporting two cases of bilateral lung transplant (LTx) recipients with comorbidities, including chronic lung allograft dysfunction (CLAD), showed serum trough voriconazole concentrations mostly between 100 and 200 ng/mL, when quantifiable [18]. These values, even considering the differences in terms of administered inhaled dose are significantly higher than serum through voriconazole concentrations determined in the present study. Other reasons that might justify the observed differences, are the device, although similar, or the dry powder formulation obtained with a different engineering technology.

Overall, inhaled voriconazole was safe and well tolerated in healthy subjects and subjects with mild to moderate asthma at doses up to 40 mg administered either as a single or repeat doses for up to 10 days. Local tolerability was acceptable with some subjects experiencing mild chest tightness that mostly resolved within few minutes post-inhalation without any treatment. There were no noticeable effects on lung function, vital signs, ECG parameters, laboratory parameters, physical examination or bronchoconstriction and asthma exacerbation.

Although the study provides valuable information on the safety and tolerability of inhaled voriconazole and serum PK, a limitation is the lack of conclusive sputum PK data due to the small number of samples obtained and the high intersubject variability observed. Collection of adequate sputum samples from healthy volunteers and subjects with mild to moderate asthma is essential for drawing conclusions on the effective delivery of voriconazole to the lungs.

Conversely, an interesting point of the present study is the determination of N-oxide voriconazole concentrations. Reports have indicated that systemic administration of voriconazole and the determination of N-oxide voriconazole concentrations can inform on the capacity of a patient to metabolize voriconazole which may be beneficial for therapeutic drug monitoring in specific patients [19]. The successful development and validation of a bioanalytical method for the simultaneous determination of

voriconazole and N-oxide voriconazole in serum and sputum samples collected during the current study, encourages the implementation of N-oxide quantitation as a practical routine to support dose adjustment, optimization of the treatment and speculation on the role of metabolism following different routes of administration.

In summary, this is the first report of the PK of inhaled doses of voriconazole in a well-designed Phase I clinical trial. The findings of the current study suggest the newly developed dry powder inhaled formulation of voriconazole is safe and well tolerated, with low systemic exposure and limited accumulation following repeat administration in the investigated dose range. Further studies are needed to understand if an inhaled formulation of voriconazole that provides efficient delivery to the lung, while minimizing systemic exposure compared to oral administration would represent an improved treatment approach for ABPA.

Author Contributions

Daniele Colombo and Federica Sala wrote the manuscript. Dave Singh and Giovanni Caponetti contributed to study design and conceptualization. Federica Sala contributed to data analysis. Elena Tiberio and Antonio Cervetti contributed to study management and data collection. All authors reviewed and provided comments on the manuscript and read and approved the final manuscript.

Ethics Statement

This manuscript is in keeping with the ethical standards for research of the Pharmacology Research and Perspectives.

Conflicts of Interest

A. Cervetti, D. Colombo, F. Sala, E. Tiberio are current employees of Zambon. G. Caponetti is the inventor of the patents WO2022/1223009 "Method for manufacturing an inhalable powder comprising Voriconazole" and "Inhalable powder comprising Voriconazole in crystalline form" WO2022/123029. D. Singh has received consultancy fees from Aerogen, AstraZeneca, BIAL, Boehringer Ingelheim, Chiesi, Cipla, CSL Behring, EpiEndo, Genentech, GlaxoSmithKline, Glenmark, Gossamer Bio, Kinaset Therapeutics, Menarini, Novartis, Orion, Pulmatrix, Sanofi, Teva, Theravance Biopharma, and Verona Pharma.

Data Availability Statement

The research data described in this manuscript are not shared.

Principal Investigator Statement

The authors confirm that the Principal Investigator for this paper is Professor Sukh Dave Singh, MD and that he had direct clinical responsibility for patients.

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