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ORIGINAL ARTICLE

A phase 1/1b study of PUR1900, an inhaled formulation of itraconazole, in healthy volunteers and asthmatics to study safety, tolerability and pharmacokinetics

David L. Hava[1] | Lisa Tan[2] | Patrick Johnson[3] | Aidan K. Curran[1] | Jason Perry[1] | Steve Kramer[1] | Katie Kane[1] | Pauline Bedwell[4] | Gary Layton[5] | Clarie Swann[6] | Dennis Henderson[6] | Naimat Khan[7] | Lucy Connor[7] | Litza McKenzie[6] | Dave Singh[7,8] | James Roach[1]

[1]Pulmatrix Inc, Lexington, MA, USA

[2]Lisa Tan Pharma Consulting, Kingston, Cambridge, UK

[3]JPharma Solutions, Bulach, Switzerland

[4]Way Hill, Minister, Kent, UK

[5]ParamStat Limited, Canterbury, Kent, UK

[6]Quotient Clinical, Nottingham, UK

[7]Medicines Evaluation Unit, The Langley Building, Wythenshawe Hospital, Wythenshawe, UK, England

[8]University of Manchester, Manchester University NHS Hospital Trust, Manchester, UK

Correspondence
David L. Hava, Pulmatrix Inc, 99 Hayden Ave, Suite 390, Lexington, MA 02421, 781-357-2333.
Email: dave.hava@gmail.com

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Pulmatrix Inc.

Aims: Oral itraconazole has variable pharmacokinetics and risks of adverse events associated with high plasma exposure. An inhalation formulation of itraconazole (PUR1900) is being developed to treat allergic bronchopulmonary aspergillosis, an allergic inflammatory disease occurring in asthmatics and patients with cystic fibrosis.

Methods: A 3-part, open-label Phase 1 study was conducted to evaluate safety, tolerability and pharmacokinetics of PUR1900. Healthy volunteers (n = 5–6/cohort) received either single (Part 1) or multiple (Part 2) ascending doses of PUR1900 for up to 14 days. In Part 3 stable, adult asthmatics received a single dose of 20 mg PUR1900 or 200 mg of oral Sporanox (itraconazole oral solution) in a 2-period randomized cross-over design. Itraconazole plasma and sputum concentrations were evaluated.

Results: None of the adverse events considered as at least possibly related to study treatment were moderate or severe, and none were classed as serious. The most common was the infrequent occurrence of mild cough. Itraconazole plasma exposure increased with increasing doses of PUR1900. After 14 days, PUR1900 resulted in plasma exposure (area under the concentration–time curve up to 24 h) 106- to 400-fold lower across doses tested (10–35 mg) than steady-state exposure reported for oral Sporanox 200 mg. In asthmatics, PUR1900 geometric mean maximum sputum concentrations were 70-fold higher and geometric mean plasma concentrations were 66-fold lower than with oral Sporanox.

Conclusion: PUR1900 was safe and well-tolerated under the study conditions. Compared to oral dosing, PUR1900 achieved higher lung and lower plasma exposure. The pharmacokinetic profile of PUR1900 suggests the potential to improve upon the efficacy and safety profile observed with oral itraconazole.

KEYWORDS

allergy, asthma, infectious disease, Phase 1

The authors confirm that the PIs for this paper were Dr McKenzie and Dr Singh and that they had direct clinical responsibility for patients.

1 | INTRODUCTION

Aspergillus spp. are spore-forming moulds that cause significant morbidity and mortality in a number of patient populations. *Aspergillus fumigatus* causes chronic infection in patients with chronic lung diseases such as asthma and cystic fibrosis. In asthma patients, fungal colonization and infection can result in allergic bronchopulmonary aspergillosis (ABPA), a complex T-helper type 2 hypersensitivity reaction marked by high levels of eosinophils and IgE, and antibodies reactive with *Aspergillus* antigens.[1,2] Through repeated cycles of infection and inflammation, patients with ABPA are at high risk for frequent exacerbations and the development of bronchiectasis. ABPA management focuses on using oral corticosteroids to suppress inflammation and oral antifungals to attempt to eradicate *A. fumigatus* from the airways.[3]

Itraconazole is the most commonly used antifungal therapy and randomized controlled trials have demonstrated efficacy in treating ABPA either as adjunctive or monotherapy[4-6]; however, oral doses of itraconazole have variable absorption and food interactions, and no correlation is observed between serum and sputum levels.[7] Patients with cystic fibrosis have highly variable pharmacokinetics and steady-state sputum concentrations of itraconazole often fall below the minimum inhibitory concentration (MIC_{90}) for *A. fumigatus* in most subjects.[8,9] High plasma concentrations of itraconazole can lead to significant drug–drug interactions (DDI) and significantly impact the pharmacokinetics of other drugs[10] through inhibition of **cytochrome p450 3A4** in the liver and increasing plasma exposure has been linked to a greater probability for adverse events (AEs).[11] Poor pharmacokinetics and risks associated with its side effect profile limit itraconazole therapeutic efficacy.[7]

Inhaled anti-infective therapies have been developed to treat local pulmonary infections caused by a number of different pathogens in patients with chronic respiratory diseases.[12] PUR1900 is a dry powder formulation of itraconazole formulated in a particle engineered dry powder platform called iSPERSE, which allows highly efficient delivery of high drug loads to the lungs via inhalation.[13,14] PUR1900 is engineered to have a small aerodynamic particle size for efficient pulmonary delivery with the goal of achieving high lung concentrations of itraconazole to treat pulmonary infections. A Phase 1 study was conducted to assess the safety and tolerability of PUR1900 in healthy subjects and subjects with stable mild-to-moderate asthma, and to characterize the single and multiple dose pharmacokinetics of itraconazole following inhalation.

2 | METHODS

2.1 | Study drug

PUR1900 is a dry powder for oral inhalation comprising itraconazole as the active pharmaceutical ingredient. PUR1900 was administered with a reloadable, single-capsule passive dry powder inhaler (DPI; RS01 Model 7; Plastiape S.p.A., Osnago, Italy). Each PUR1900 capsule contains 5 mg of itraconazole. Increasing doses of itraconazole were achieved by administering increasing numbers of capsules. All doses refer to the nominal dose of itraconazole.

2.2 | Study design part 1 and part 2: Healthy volunteers

An integrated Phase 1, multicentre, 3-part, open-label study in healthy adult subjects and adult subjects with mild-to-moderate stable asthma was conducted to evaluate the safety, tolerability and pharmacokinetics of PUR1900. The study was approved by the local ethics committee and the Medicines and Healthcare products Regulatory Agency (MHRA). All subjects provided written informed consent (clinicaltrials.gov NCT03479411).

Part 1 enrolled 23 healthy subjects in 4 separate cohorts who received single doses of 5, 10, 25 and 35 mg PUR1900. Safety and tolerability evaluations, including spirometry and electrocardiograms, were performed predose, and 0.5, 2, 4, 8, 12 and 24 h after dosing. Additional safety assessments and blood collections were performed 48 and 96 h after dosing and at follow-up.

Part 2 enrolled 18 healthy subjects in 3 separate cohorts who received daily doses of 10, 20 and 35 mg PUR1900 for 14 days. Safety and tolerability were made on Day 1 and Day 14 were evaluated on each day of the study as described in Part 1. Additional safety and pharmacokinetic assessments were performed 96 and 168 h after the last dose and at follow up.

2.3 | Study design part 3: Mild-to-moderate asthmatics

Part 3 was a 2-period, randomised, crossover study in adult subjects (n = 17) with mild-to-moderate stable asthma who received single doses of PUR1900 or itraconazole oral solution (Sporanox Oral Solution; 10 mg/mL). Eligible subjects were GINA STEP 2 or 3 patients[15] and required treatment with either inhaled corticosteroids (ICS) or ICS plus a long-acting β-agonist. Enrolled subjects did not have ABPA. Additionally, eligible subjects had a pre-bronchodilator forced expiration volume in 1 s (FEV_1) ≥ 70% of predicted normal at screening and needed to produce a sputum sample at screening of a quality required for drug concentration assessments. Eligible subjects were randomised to receive a single oral dose of 200 mg Sporanox or a single inhaled PUR1900 dose of 20 mg in Period 1. After a washout of at least 14 days, each subject received the alternative treatment in Period 2. All 17 subjects received 200 mg Sporanox (9 in sequence 1 and 8 in sequence 2), and 16 subjects received 20 mg PUR1900. One subject was withdrawn from the study prior to dosing in Period 2 due to a positive drug test and did not receive PUR1900. Safety and tolerability were performed as in Part 1.

2.4 | Pharmacokinetic sampling and analysis

In Part 1 and 3, blood was collected predose and 0.25, 0.75, 1.5, 2, 3, 4, 6, 8, 12, 16 and 24 h after inhalation to determine plasma concentrations of itraconazole and the active metabolite, hydroxy-itraconazole using a validated liquid chromatography–tandem mass spectrometry method with an lower limit of quantitation of 0.1 ng/mL for each analyte. Additional blood collections were performed 48 h and 96 h after dosing and at follow-up. For itraconazole the mean intrarun precision ranged from 15.5 to 8.5 (% coefficient of variance [%CV]) and the mean inter-run precision ranged from 17.4 to 8.7 (%CV). For hydroxy-itraconazole the same measures were 11.4 to 2.9 (%CV) and 19.7 to 4.1 (%CV) for the inter-run and intrarun precision, respectively.

In Part 2, blood collections were made on Day 1 and Day 14 as described in Part 1 and were additionally performed predose on Day 2–13. Additional assessments were performed 96 h and 168 h after the last dose and at follow up.

Sputum induction was performed in Part 3 to assess lung drug concentrations 2, 6, 23, 48 and 96 h after dosing in accordance with European Respiratory Society guidelines.[15] Subjects received 400 μg **salbutamol** orally inhaled via a spacer and spirometry was performed approximately 20 min after administration. Subjects then inhaled nebulized hypertonic saline for 5 min on up to 3 occasions. After each inhalation period, subjects were asked to expectorate sputum for analysis. Sputum levels of itraconazole and hydroxy-itraconazole were determined by a liquid chromatography–tandem mass spectrometry method validated for sputum over a low (0.1--100 ng/mL) and high (50–10000 ng/mL) range. For itraconazole low range method, the mean intrarun precision ranged from 3.9 to 9.2 (%CV) and the mean inter-run precision ranged from 4.4% to 12.3% (%CV). For hydroxy-itraconazole the intrarun precision was 3.4 to 9.5% (%CV) and the inter-run precision was 4.1 to 9.8 (%CV). Results were similar for the high range method: itraconazole intrarun precision was 2.8 to 5.5 (%CV), itraconazole inter-run precision was 2.7–10.9 (%CV), hydroxy-itraconazole intrarun precision was 2.8–8.2 (%CV), and itraconazole inter-run precision was 2.9 to 11.3 (%CV).

2.5 | Populations and statistical analysis

In each study part, the safety population included all subjects who received at least 1 dose of study medication and the pharmacokinetic population included all subjects in the safety population who had at least 1 evaluable postdose pharmacokinetic measurement. Noncompartmental pharmacokinetic parameters of itraconazole and hydroxy-itraconazole were calculated for each subject for each treatment. Noncompartmental analysis was performed using PKNCA (version 0.8.4) with R (version 3.4.3)[16] and statistical analyses were performed using SAS (version 9.4). In Part 2, accumulation ratios were calculated from Day 1 and Day 14 area under the concentration–time curve up to 24 h ($AUC_{0\text{-}24h}$) and maximum concentration (C_{max}) values. Dose proportionality was assessed by combining data from Cohorts 1 and 2 (presented) and within each cohort (not presented).

Relative bioavailability of PUR1900 and Sporanox were determined in Part 3. Pharmacokinetic parameters underwent a natural logarithmic transformation and were analysed using a linear mixed model including terms for treatment, period and sequence fitted as fixed effects and subject nested within sequence fitted as a random effect. PUR1900 (20 mg) C_{max} and $AUC_{0\text{-}24h}$ were also compared between asthmatics (Part 3) and healthy volunteers (Part 2) using analysis of variance. The difference between adjusted means and the associated 90% confidence interval (CI) obtained from the model were back transformed from the log scale to obtain the adjusted geometric mean ratios (GMRs) and corresponding 90%CI for the ratio.

3 | RESULTS

3.1 | Patients and demographics

Twenty-three subjects (10:13 male:female) aged 19–60 were dosed in Part 1 and 18 subjects (14:4 male:female) aged 21–60 were dosed in Part 2. The majority of subjects were white (78.3% in Part 1 and 83.3% in Part 2) and all subjects were within the reference range for body mass index (18–35 kg/m^2). Seventeen subjects aged 18–55 years were enrolled in Part 3 (10:7 male:female). Subjects in Part 3 had a confirmed diagnosis of asthma, were on treatment with either ICS or ICS plus long-acting β-agonist and met GINA Step 2 or 3 criteria.[15] CONSORT diagrams are shown in Figure 1 and Figure 2, study demographics are shown in Table 1.

3.2 | Single-dose pharmacokinetics in healthy subjects

After single doses of PUR1900, absorption of itraconazole into plasma was rapid at all dose levels. All subjects had quantifiable plasma concentrations between 0.25 and 48 h and at doses ≥10 mg exposure was observed through 96 h. Plasma exposure was generally maintained at similar levels over the first 24 h indicating sustained absorption (Figure 3a). Beyond 24 h, plasma concentrations declined in a steady mono-exponential manner and the median rate of elimination for itraconazole was similar across doses (Table 2). Kinetics of hydroxy-itraconazole exposure were similar to itraconazole, with lower hydroxy-itraconazole exposure relative to itraconazole at all doses (Supplemental Table S1).

3.3 | Multiple-dose pharmacokinetics in healthy subjects

In Part 2, PUR1900 single dose pharmacokinetics were similar to Part 1 (Supplemental Table 2) and the kinetics of exposure were similar on

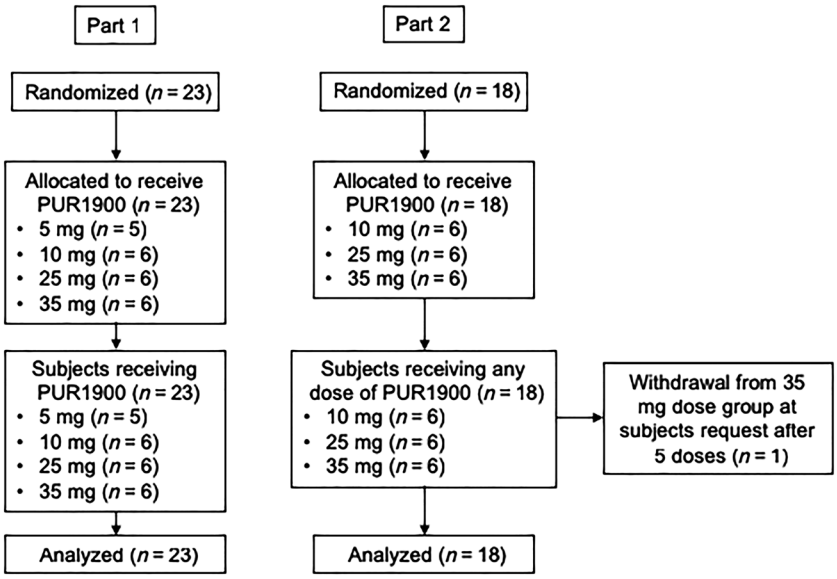

FIGURE 1 CONSORT diagram for Parts 1 and 2 of the study

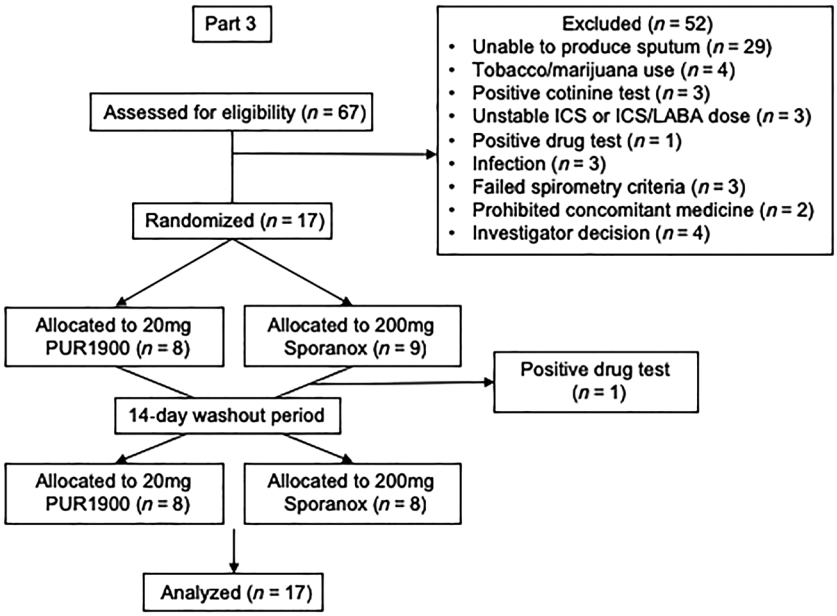

FIGURE 2 CONSORT diagram for Part 3 of the study

TABLE 1 Study demographics

	Part 1 (n = 23)		Part 2 (n = 18)		Part 3 (n = 17)	
	Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range
Age (y)	35.3 (13.3)	19–60	42.9 (13.7)	21–60	38.8 (11.1)	18–55
Height (cm)	169.7 (9.52)	152–184	171.7 (5.33)	159–178	173.2 (9.49)	154–187
Weight (kg)	78.5 (14.1)	55.4–112	80.8 (12.6)	64–102	82.1 (13.9)	59.5–107.7
BMI (kg/m2)	27.2 (3.71)	20.9–34.8	27.4 (3.83)	22.7–34.9	27.3 (3.01)	24.0–32.3
FEV_1 (L)	n/a	n/a	n/a	n/a	3.50 (0.87)	2.36–5.20
Subjects taking ICS	n/a	n/a	n/a	n/a	12	
Subjects taking ICS/LABA	n/a	n/a	n/a	n/a	5	
Male:Female	10: 13		14: 4		10: 7	

BMI, body mass index; FEV_1, forced expiration volume in 1 s; ICS, inhaled corticosteroids; LABA, long-acting β-agonist; n/a = not applicable; SD, standard deviation

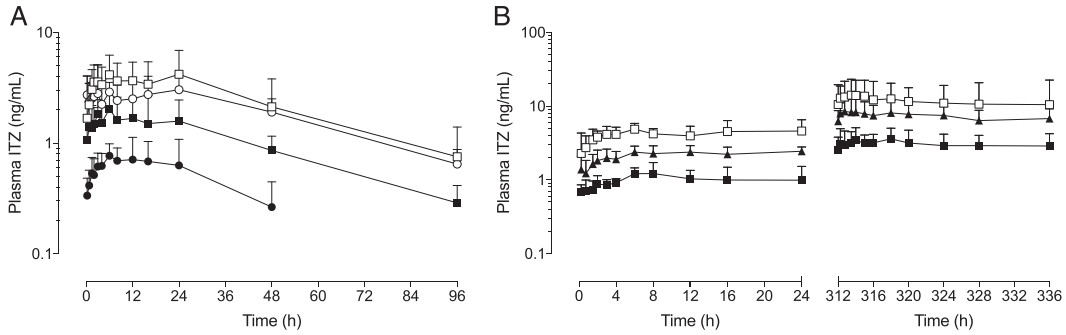

FIGURE 3 Pharmacokinetics of itraconazole in healthy subjects. (A) Itraconazole plasma levels were determined after single doses of PUR1900 for up to 96 h after dosing (part 1). Data depict geometric mean (+ geometric standard deviation) concentrations for 5 mg PUR1900 (●), 10 mg PUR1900 (■), 25 mg PUR1900 (○), and 35 mg PUR1900 (□). (B) Itraconazole plasma levels were determined after single daily doses of PUR1900 for 14 days (part 2). Data depict the day 1 and day 14 geometric mean (+ geometric standard deviation) concentrations for 10 mg PUR1900 (■), 20 mg PUR1900 (▲), and 35 mg PUR1900 (□)

TABLE 2 Itraconazole single (SAD) and multiple (MAD) ascending dose pharmacokinetics

	Dose (mg)	T_{max} (h)	C_{max} (ng/mL)	$AUC_{0\text{-}24h}$ (ng h/mL)	Kel (1/h)	Cl/F (L/h)	Vz/F (L)	Accumulation ratio C_{max}	Accumulation ratio $AUC_{0\text{-}24h}$
Part 1 SAD day 1	5	6	0.873 (35.4)	15.9 (36.5)	0.0298	166 (36.0)	5130 (43.6)	n/a	n/a
	10	6	2.28 (26.8)	38.9 (43.1)	0.0194	90.8 (31.0)	4020 (58.1)	n/a	n/a
	25	3	3.90 (38.2)	64.9 (30.6)	0.0210	118 (26.5)	5550 (31.9)	n/a	n/a
	35	18	4.58 (48.4)	86.9 (42.6)	0.0230	192 (9.7)	7920 (7.3)	n/a	n/a
Part 2 MAD day 14	10	5	3.77 (34.2)	73.2 (35.1)	0.0169	n/d	n/d	2.9	3.0
	20	4	8.98 (37.9)	175 (32.7)	0.0197	n/d	n/d	3.4	3.3
	35	0.75	15.2 (49.3)	276 (62.2)	0.0170	n/d	n/d	3.0	2.8
Part 3 day 1	PUR1900 20 mg	4	2.5 (58.5)	45.3 (64.0)	0.0248	182 (76.3)	7350 (67.8)	n/a	n/a
	Sporanox 200 mg	1.5	606 (37.6)	3660 (27.6)	0.0200	28.8 (33.8)	1440 (32.7)	n/a	n/a

Data are geometric means (% coefficients of variance); except medians are presented for T_{max} and Kel.
$AUC_{0\text{-}24h}$, area under the concentration–time curve up to 24 h; C_{max}, maximum concentration; Kel, rate of elimination; n/a = not applicable; n/d = not determined; T_{max}, time to C_{max}

both Day 1 and Day 14 (Figure 3b). Plasma concentrations of itraconazole and hydroxy-itraconazole increased with each repeat dose, with concentrations at or close to steady state by Day 14. Itraconazole accumulation was consistent across dose levels, ranging from 2.9- to 3.4-fold accumulation based on C_{max} and 2.8- to 3.3-fold accumulation based on $AUC_{0\text{-}24h}$ (Table 2). Levels of hydroxy-itraconazole were lower relative to itraconazole after 14 days of dosing, with similar levels of accumulation observed (Supplemental Table 3). After 14 days of dosing, plasma itraconazole and hydroxy-itraconazole concentrations declined in a steady mono-exponential manner, with a similar rate of elimination across all doses and both parent and metabolite (Table 2 and Supplemental Table 3).

Using the combined single dose data from Part 1 and Part 2, estimates of β (90% CI) were 0.87 (0.73, 1.00) and 0.89 (0.76, 1.02) for C_{max} and $AUC_{0\text{-}24h}$, respectively, indicating that systemic exposure (both peak and overall) increased with increasing dose of PUR1900 in a broadly dose proportional manner (Figure 4).

3.4 | Pharmacokinetics in asthma patients

After inhaled dosing, 14/16 subjects had quantifiable plasma itraconazole concentrations up to 96 h and itraconazole was quantifiable in all samples up to 96 h following oral dosing. PUR1900 resulted in similar plasma exposure in asthmatics and healthy volunteers. The GMRs (90%CI) of C_{max} and $AUC_{0\text{-}24h}$ for asthmatics compared to healthy volunteers following inhalation of 20 mg PUR1900 were 94.59% (63.80, 140.24%) and 85.27% (55.74, 130.46%), respectively.

Following oral and inhaled doses, itraconazole was quickly absorbed into the systemic circulation with median time to C_{max} estimates of 4.0 and 1.5 h for PUR1900 and Sporanox, respectively (Table 2). Following PUR1900 administration, itraconazole and hydroxy-itraconazole plasma exposure generally were maintained over the first 24 h, indicating a prolonged absorption, albeit both at very low levels (Figure 5a). In contrast, oral itraconazole was rapidly absorbed and eliminated, such that itraconazole exposure peaked

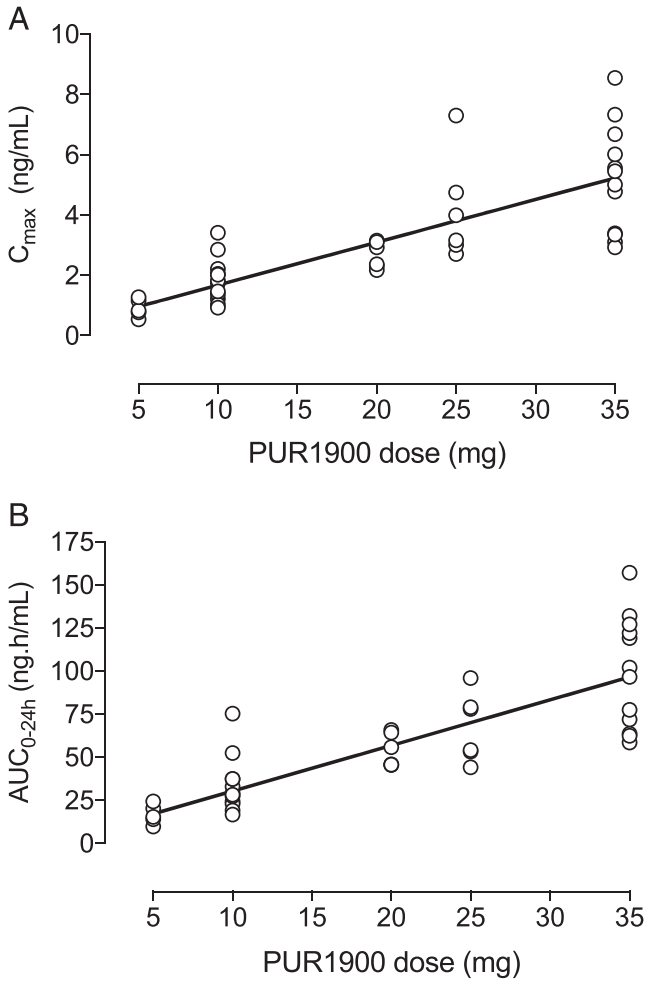

FIGURE 4 Relationship between dose and itraconazole exposure in healthy subjects. (A) maximum concentration (C_{max}) or (B) area under the concentration–time curve up to 24 h ($AUC_{0\text{-}24h}$) for each subject after receiving a single dose of PUR1900 in part 1 and part 2 of the study were plotted against the administered PUR1900 dose

within 1.5–3 h after dosing, and rapidly declined to levels that were 15% of C_{max} 12 h after dosing (Figure 5a). Hydroxy-itraconazole showed a longer exposure, peaking at 1.5–6 h after dosing and maintained exposure at 41% of C_{max} 24 h after dosing. Similar to itraconazole, hydroxy-itraconazole levels were lower following inhaled dosing compared to oral dosing (Supplemental Table 4).

Adjusted geometric mean systemic itraconazole exposure over 24 h ($AUC_{0\text{-}t}$) after PUR1900 was 1.51% of the exposure after oral dosing, approximately 66-fold lower. For hydroxy-itraconazole, the adjusted geometric mean plasma $AUC_{0\text{-}t}$ after PUR1900 was 0.32% of the exposure after oral dosing, approximately 310-fold lower. Consistent with these differences, the relative bioavailability of itraconazole was substantially lower following PUR1900 inhalation compared to oral dosing. The GMRs (90%CI) for C_{max}, $AUC_{0\text{-}t}$ and $AUC_{0\text{-}inf}$ were 0.41% (0.33%, 0.51%), 1.51% (1.19%, 1.91%) and 1.62% (1.27%, 2.06%), respectively. A lower percentage of the $AUC_{0\text{-}inf}$ was extrapolated for oral Sporanox (median 7.2%; range 0.1–25%) compared to PUR1900 (median 10.2%; range 7.7–35%).

In contrast to plasma levels, high sputum itraconazole exposure was observed following PUR1900 (20 mg) dosing relative to oral dosing (200 mg). PUR1900 geometric mean C_{max} was 70-fold higher than C_{max} after oral dosing (4530 ng/mL *vs* 65.4 ng/mL, respectively) and levels remained high through 23 h (Figure 5b). At 2 and 6 h after PUR1900 dosing, most subjects produced sputum itraconazole concentrations greater than the MIC_{90} of itraconazole against *A. fumigatus*, and 40% of subjects exceeded this concentration at 23 h (Figure 5c). In contrast, only 13% of orally dosed subjects had itraconazole concentrations above the MIC_{90} at the 2 h timepoint, with none of the subjects above the MIC_{90} at 6 h after oral dosing. Sputum hydroxy-itraconazole values were very low following both PUR1900 and Sporanox, with most sputum samples following PUR1900 inhalation having concentrations that were not quantifiable.

3.5 | PUR1900 safety and tolerability

In Part 1, 1 subject experienced 2 serious AEs (appendicitis and peritonitis) unrelated to PUR1900 that occurred 7 days after dosing. No other serious AEs were reported. There were 26 AEs in Part 1 (Table 3), with no dose-related trend in the incidence of AEs except for an increased incidence of cough in the 25 mg (66.7%) and 35 mg (66.7%) PUR1900 cohorts. In Part 2, there were 36 AEs (Table 3). One subject received 35 mg PUR1900 for 5 days prior to withdrawing consent. Eight (44.4%) subjects experienced a mild adverse drug reaction (ADR) of cough; 2, 3 and 3 subjects in the 10, 20 and 35 mg PUR1900 cohorts, respectively. In all cases, cough resolved spontaneously in seconds to minutes after dosing and required no intervention. In addition, 1 (16.7%) subject in the 10 mg cohort experienced a mild ADR of chest discomfort.

After a single dose of PUR1900 or oral Sporanox in asthma patients, all AEs considered as at least possibly related to study drug were characterized as mild. Overall, 6 (35.3%) subjects experienced 7 AEs following a single dose of Sporanox and 11 (68.8%) subjects experienced 16 AEs following 20 mg PUR1900 (Table 4). Three subjects experienced a mild ADR of cough during dosing and 1 subject experienced a mild ADR of chest discomfort following PUR1900. In all cases, cough resolved spontaneously in seconds to minutes after dosing and required no intervention. There were no clinically significant changes in ECGs or vital signs. One subject had an increase in muscle enzymes unrelated to treatment following PUR1900.

In Part 1, a ≥10% decrease in FEV_1 from baseline (predose Day 1) at any timepoint was observed in 2/6 subjects in the 10 mg PUR1900 cohort (at 12 h postdose in 1 subject and at 12 and 24 h postdose in the second subject) and in 2/6 subjects in the 35 mg cohort (at the follow up visit in 1 subject and at 24 h postdose in the second subject). In Part 2, a ≥10% decrease in FEV_1 from baseline was observed at any timepoint in 4 subjects; 1/6 subjects in the 10 mg PUR1900 cohort, 2/6 subjects in the 20 mg cohort and 1/6 subjects in the 35 mg cohort. These FEV_1 declines occurred at

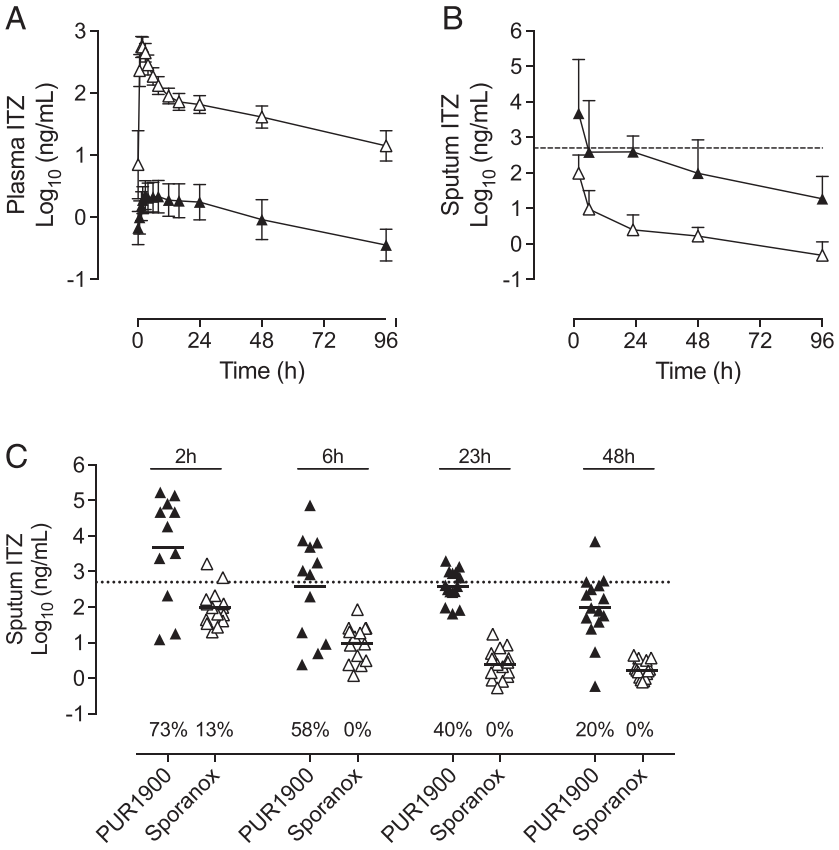

FIGURE 5 Plasma and sputum pharmacokinetics of itraconazole in asthmatics. Itraconazole (A) plasma levels or (B) sputum levels were determined after single doses of PUR1900 or oral Sporanox. Geometric mean (+geometric standard deviation) of itraconazole levels are shown; 20 mg PUR1900 (▲) or 200 mg Sporanox (△). The number of sputum samples collected per timepoint was: 11 (2 h), 12 (6 h), 15 (23 h), 15 (48 h), and 14 (96 h) for PUR1900 and 15 (2 h), 13 (6 h), 15 (23 h), 15 (48 h), 16 (96 h) for Sporanox. (C) Itraconazole sputum concentrations for each subject are shown for 20 mg PUR1900 (▲) or 200 mg Sporanox (△). The geometric mean is indicated by a line and the percentage of subjects above the MIC_{90} at each time point are shown. The dotted line at 500 ng/mL in B and C indicates the MIC_{90} for *Aspergillus fumigatus*[17]

TABLE 3 Incidence of treatment-emergent adverse events (TEAEs) in healthy subjects

	Part 1 – single ascending dose					Part 2 – multiple ascending dose			
	5 mg (*n* = 5)	10 mg (*n* = 6)	25 mg (*n* = 6)	35 mg (*n* = 6)	Overall (*n* = 23)	10 mg (*n* = 6)	20 mg (*n* = 6)	35 mg (*n* = 6)	Overall (*n* = 18)
Subjects reporting TEAEs	2 (3)	2 (8)	5 (11)	4 (4)	13 (26)	2 (4)	5 (19)	5 (13)	12 (36)
Respiratory, thoracic and mediastinal disorders	0	1 (1)	4 (4)	4 (4)	9 (9)	2 (3)	4 (14)	4 (7)	10 (24)
Cough	0	0	4 (4)	4 (4)	8 (8)	2 (3)	3 (12)	3 (6)	8 (21)
Epistaxis	0	1 (1)	0	0	1 (1)	0	1 (2)	1 (1)	2 (3)
Musculoskeletal and connective disorders	2 (3)	0	1 (2)	0	3 (5)	0	1 (1)	1 (1)	2 (2)
Gastrointestinal disorders	0	1 (1)	1 (1)	0	2 (2)	0	0	0	0
Injury, poisoning, and procedural complications	0	1 (2)	1 (1)	0	2 (3)	0	0	0	0
Nervous system disorders	0	0	2 (2)	0	2 (2)	0	1 (1)	2 (3)	3 (4)
Skin and subcutaneous tissue disorders	0	1 (1)	1 (1)	1 (1)	2 (2)	0	0	0	0
Renal and urinary disorders	0	0	0	0	0	0	1 (1)	0	1 (1)
Infections and infestations	0	1 (3)	0	0	1 (3)	0	1 (1)	0	1 (1)
General disorders and administration site conditions	0	0	0	0	0	1 (1)	0	2 (2)	3 (3)
Eye disorders	0	0	0	0	0	0	1 (1)	0	1 (1)

Data show the no. of subjects (no. of events) that experienced each TEAE.

various timepoints across the 14 days of dosing in these 4 subjects, with no obvious pattern associated with either dose received or timepoint in which the declines were observed. In no instance was a decline in FEV_1 associated with any symptoms, nor considered to be an AE. In Part 2, all FEV_1 values were within 10% of baseline at the final follow-up visit.

TABLE 4 Incidence of treatment-emergent adverse events (TEAEs) in asthmatics

	Sporanox 200 mg (*n* = 17)	PUR1900 20 mg (*n* = 16)
Subjects reporting TEAEs (number of events)	6 (7)	11 (16)
Respiratory, thoracic and mediastinal disorders	1 (1)	4 (4)
Cough	0	3 (3)
Chest discomfort	0	1 (1)
Wheezing	1 (1)	0
Nervous system disorders	2 (2)	4 (5)
Skin and subcutaneous tissue disorders	2 (2)	3 (3)
Immune system disorders	0	2 (2)
General disorders and administration site conditions	1 (1)	0
Investigations	0	1 (1)
Psychiatric disorders	1 (1)	0

Data represent the number of subjects and number of events in brackets for each TEAE

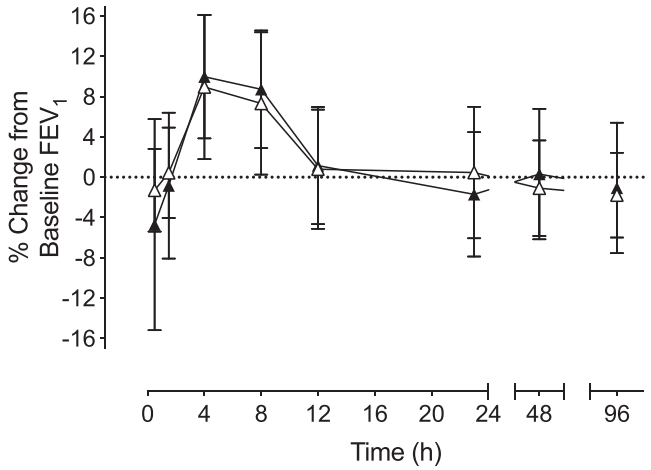

FIGURE 6 Change in forced expiration volume in 1 s (FEV_1) in asthmatics following PUR1900 and oral Sporanox. Spirometry was performed in mild-to-moderate asthmatics for up to 96 h after dosing with 20 mg PUR1900 or 200 mg oral Sporanox. The percent change from baseline FEV_1 was calculated. The mean ± standard deviation are shown following 20 mg PUR1900 (*n* = 16; ▲) or 200 mg Sporanox (*n* = 17; △)

In Part 3, spirometry was measured up to 96 h after dosing. As part of the sputum induction procedure, subjects received a short-acting β-agonist prior to sputum induction (at 2, 6, 23, 48 and 96 h postdose) and in these instances, spirometry was performed prior to short-acting β-agonist use. A ≥10% decrease in FEV_1 from baseline was observed in 5/16 subjects in at least 1 time point following administration of PUR1900, including 2 subjects in which an FEV_1 decline of ≥10% was observed at 0.5 and 1.5 h postdose. In the first subject, the decline in FEV_1 observed at 0.5 (34% decrease) and 1.5 (23% decrease) h postdose was associated with mild chest discomfort and wheezing and was considered an AE. This subject did not require the administration of rescue medication, and following administration of 4 puffs of salbutamol 2 h post-dosing and prior to undergoing sputum induction the subject's FEV_1 was back to baseline. This was the only subject in the study for whom a postdose decline in FEV_1 following the administration of PUR1900 was associated with an AE. In the other 3 subjects, declines in FEV_1 postdose were observed at 48 and 96 h postdose in 1 subject, at 96 h postdose in 1 subject, and at 23 h postdose in the third subject.

There was no relationship between subjects who coughed and subjects who experienced a decline in spirometry and no relationship between PUR1900 dosing and the timing of when declines in spirometry were observed. Other than a slight decrease in the mean FEV_1 observed 30 min postinhalation of PUR1900 driven by the 2/16 subjects that experienced an FEV_1 decline at this timepoint, there were no notable differences detected in mean change from baseline at any other timepoint between PUR1900 and Sporanox (Figure 6). The mean changes observed at 30 min post inhalation of PUR1900 were driven by 2 subjects who experienced declines of 23 and 34% at this timepoint. Neither subject required administration of a rescue bronchodilator and FEV_1 values comparable to predose were observed in both subjects at the 2-h time point, following administration of 400 μg of salbutamol prior to planned sputum induction.

4 | DISCUSSION

Safety and tolerability of PUR1900, an inhaled dry powder formulation of itraconazole, was assessed in healthy subjects and mild-to-moderate asthmatics. PUR1900 doses up to 35 mg itraconazole for 14 days in healthy subjects and single doses of 20 mg PUR1900 appeared to be safe and well tolerated in both study populations, although some individuals experienced a transient cough immediately after dosing. Pharmacokinetic data indicate that PUR1900 results in higher and more sustained lung exposure of itraconazole relative to oral dosing, an important factor in the activity of itraconazole against *Aspergillus*.[18] In addition, plasma exposure following PUR1900 inhalation was substantially lower than that with oral dosing, potentially decreasing risks of systemic itraconazole-related AEs and drug–drug-interactions. Finally, the sustained nature of lung exposure and the relatively low accumulation with repeat dosing supports once daily dosing in future clinical trials.

The most common ADR in healthy subjects and asthma patients was the occurrence of mild cough immediately after dosing. All coughs were mild and resolved without treatment within seconds to minutes. Coughs were not associated with any clinically significant change in spirometry parameters or symptoms of bronchoconstriction, such as wheeze or chest tightness. Cough associated with dosing is commonly observed following inhalation drugs with high powder loads and often reduces in incidence and severity with extended

dosing and acclimation.[19-21] Of note, cough associated with PUR1900 administration resolved spontaneously. In each part of the study, a small number of subjects experienced a decline in FEV_1 of ≥10% following dosing in at least 1 time point that spirometry was measured; however, in only 1 subject in Part 3 was a decline in FEV_1 associated with chest discomfort.

After both single and repeat doses of PUR1900, itraconazole was rapidly absorbed and systemic exposure of itraconazole and hydroxy-itraconazole increased with increasing dose of PUR1900 in a broadly dose proportional manner. Steady-state systemic exposure appeared to be achieved by 14 days of dosing, with very low systemic exposure of itraconazole and hydroxy-itraconazole. PUR1900 steady state $AUC_{0\text{-}24h}$ was 106- and 400-fold lower for the 35- and 10-mg doses, respectively, than that achieved with the exposure observed with twice daily dosing of oral Sporanox solution (29 271 ng h/mL) in healthy subjects in a previous study.[22] Itraconazole and hydroxy-itraconazole are potent inhibitors of cytochrome p450 3A4 and contraindicated with a number of drugs and drug classes due to risks of significant drug–drug interactions and cardiovascular events.[23] Further, the probability of AEs associated with oral itraconazole dosing increase with increasing plasma concentrations of itraconazole and therapeutic drug monitoring is important during treatment.[11,24] PUR1900 may reduce the risks of AEs and improve the therapeutic window relative to oral itraconazole due to substantially reduced plasma exposure following inhalation.

Assessment of plasma drug concentrations after inhalation can be used as a surrogate for monitoring lung exposure.[25] PUR1900 has a mass median aerodynamic diameter of ~2.5 μm and a high fine particle fraction, resulting in efficient lung delivery and low oral deposition similar to other products formulated in iSPERSE.[13,26] Following a single dose of PUR1900, the geometric mean plasma ratio of hydroxy-itraconazole to itraconazole based on $AUC_{0\text{-}t}$ was 0.345–0.474, whereas after oral itraconazole dosing, this ratio is approximately 2-fold.[22] Lower metabolite exposure is consistent with reduced hepatic metabolism of itraconazole and lower first-pass metabolism following inhalation of PUR1900. Gut absorption following oral dosing leads to rapid metabolism of the itraconazole to hydroxy-itraconazole, resulting in the higher metabolite-to-parent ratio. Lower metabolite exposure after inhalation strengthens the interpretation that lung itraconazole exposure can be assessed through the measurement of plasma levels, as the reduced metabolism of itraconazole is evidence that the plasma itraconazole levels are achieved primarily via lung absorption and are therefore reflective of the levels of itraconazole in the lung. At steady-state, the kinetics of itraconazole elimination and the magnitude of accumulation were similar across all doses. The elimination of itraconazole was similar after single and multiple doses indicating the absence of significant lung accumulation that would result in prolonged systemic exposure after the end of the inhalation dosing period secondary to a sink-like effect of accumulated itraconazole in the lung. Collectively, these data suggest that PUR1900 results in sustained lung exposure over 24 h after inhalation and that lung accumulation of the drug is similar to plasma accumulation following inhalation of doses up to 35 mg.

PUR1900 inhalation in asthma patients resulted in substantially higher sputum concentrations relative to oral dosing. Sputum samples were collected serially over 96 h to enable the generation of pharmacokinetic estimates following inhalation or oral dosing. Bronchoalveolar lavage and sampling of lung epithelial lining fluid is an alternative approach to evaluating pulmonary drug levels[27]; however, lavage sampling multiple times over 96 h was not considered due to safety and practical concerns. To assure that the quality and quantity of the sputum sample was suitable for drug concentration assessments, subjects in Part 3 were required to produce a sputum sample at screening that met pre-specified criteria. Given that the subjects in Part 3 were relatively mild asthmatics, it was not unexpected that a large percentage of screened patients were excluded due to the inability to produce a sputum sample that met acceptance criteria at screening.

For azoles, AUC in relation to MIC is the critical pharmacokinetic/pharmacodynamic relationship for predicting antifungal activity,[18] indicating the importance of the magnitude of exposure, but also the consistency of exposure over the dosing interval. At early time points after dosing, PUR1900 lung exposure was substantially higher than oral Sporanox and more variable. Less variability was observed 23 h after dosing, when high PUR1900 levels were still observed. While it is not unexpected to see more variability as exposure increases, part of the observed variability could result from the combination of variable aerosol deposition following inhalation and variable sampling from different parts of the lung following sputum induction. PUR1900 results in not only high lung exposure, but also sustained exposure for at least 23 h at levels above or near the reported 500 ng/mL MIC_{90} of itraconazole against *A. fumigatus*.[17] Importantly, data in asthma patients reflect the single dose lung and plasma exposure achieved with PUR1900 and oral dosing. Oral Sporanox is indicated for twice-daily dosing and results in ~7-fold systemic accumulation at steady-state,[28] which when considered in the context of the single dose data generated in the present study probably results in only transient lung levels above the MIC_{90} at steady-state following each dose. Similar to oral dosing and based on data from Part 2 of the study, itraconazole plasma levels accumulate ~3-fold following PUR1900. This suggests that at steady-state, a 20 mg dose PUR1900 will achieve levels above the MIC_{90} for 24 h in most subjects with once-daily dosing.

Itraconazole is recommended as a treatment for ABPA in conjunction with therapeutic drug monitoring to reduce fungal burden and the inflammatory stimulus in the lungs, in an effort to improve clinical outcomes and spare the long-term use of oral steroids.[29] This recommendation is based on data from 2 randomized, placebo-controlled studies and several case studies.[4,5,30] In patients with corticosteroid-dependent ABPA, 16-weeks of dosing with oral itraconazole significantly improved clinical response, as defined by a composite measure including a reduction in the dose of corticosteroids, decrease in the total IgE concentration, and increase in exercise tolerance and/or improvement in pulmonary function and/or improvement in pulmonary infiltrates.[4] In a second study in stable ABPA patients, oral itraconazole had a significant anti-inflammatory

benefit through a reduction in sputum eosinophils and eosinophil cationic protein after 16-weeks of dosing compared to placebo.[5] Exacerbations were also reduced with antifungal therapy. More recently, a study in treatment-naïve acute ABPA patients showed that oral itraconazole monotherapy improved clinical outcomes and FEV_1 after 6 weeks of therapy.[6] Collectively, these studies illustrate that, despite a variable pharmacokinetic profile, low bioavailability and risks of AEs at higher doses,[9,11,31,32] oral itraconazole can provide clinical benefit in ABPA.

Here, we show that lung delivery of itraconazole may offer the potential to overcome the pharmacokinetic limitations of oral itraconazole, while achieving significantly higher and prolonged lung concentrations relative to oral dosing. High lung concentrations achieved through inhalation may increase the duration that lung itraconazole levels remain above minimum inhibitory concentrations, a critical parameter of triazole efficacy.[18] Thus, inhaled itraconazole offers the possibility to improve upon both the efficacy and safety profile associated with oral itraconazole in ABPA, a disease for which there remains a significant unmet medical need. The results of this Phase 1 study support the advancement of PUR1900 into the next stage of development in patients with asthma and ABPA.

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COMPETING INTERESTS

D.L.H., A.K.C., J.P., S.K. and J.R. were employees of Pulmatrix at the time of the study and held/hold stock or stock options in Pulmatrix. The study was funded by Pulmatrix.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study have not been made openly available.

ORCID

David L. Hava https://orcid.org/0000-0002-9200-1566
Dave Singh https://orcid.org/0000-0001-8918-7075

REFERENCES

1. Moss RB. Treatment options in severe fungal asthma and allergic bronchopulmonary aspergillosis. *Eur Respir J*. 2014;43(5):1487-1500.
2. Patterson K, Strek ME. Allergic bronchopulmonary aspergillosis. *Proc am Thorac Soc*. 2010;7(3):237-244.
3. Patterson R, Greenberger PA, Radin RC, Roberts M. Allergic bronchopulmonary aspergillosis: staging as an aid to management. *Ann Intern Med*. 1982;96(3):286-291.
4. Stevens DA, Schwartz HJ, Lee JY, et al. A randomized trial of itraconazole in allergic bronchopulmonary aspergillosis. *N Engl J Med*. 2000;342(11):756-762.
5. Wark PA, Hensley MJ, Saltos N, et al. Anti-inflammatory effect of itraconazole in stable allergic bronchopulmonary aspergillosis: a randomized controlled trial. *J Allergy Clin Immunol*. 2003;111(5):952-957.
6. Agarwal R, Dhooria S, Singh Sehgal I, et al. A randomized trial of Itraconazole vs prednisolone in acute-stage allergic bronchopulmonary Aspergillosis complicating asthma. *Chest*. 2018;153(3):656-664.
7. Chmiel JF, Aksamit TR, Chotirmall SH, et al. Antibiotic Management of Lung Infections in cystic fibrosis. II. Nontuberculous mycobacteria, anaerobic bacteria, and fungi. *Ann am Thorac Soc*. 2014;11(8):1298-1306.
8. Boogaerts MA, Verhoef GE, Zachee P, Demuynck H, Verbist L, de Beule K. Antifungal prophylaxis with itraconazole in prolonged neutropenia: correlation with plasma levels. *Mycoses*. 1989;32(Suppl 1):103-108.
9. Sermet-Gaudelus I, Lesne-Hulin A, Lenoir G, Singlas E, Berche P, Hennequin C. Sputum itraconazole concentrations in cystic fibrosis patients. *Antimicrob Agents Chemother*. 2001;45(6):1937-1938.
10. Vishwanathan K, Dickinson PA, So K, et al. The effect of itraconazole and rifampicin on the pharmacokinetics of osimertinib. *Br J Clin Pharmacol*. 2018;84(6):1156-1169.
11. Lestner JM, Roberts SA, Moore CB, Howard SJ, Denning DW, Hope WW. Toxicodynamics of itraconazole: implications for therapeutic drug monitoring. *Clin Infect Dis*. 2009;49(6):928-930.
12. Weers J. Inhaled antimicrobial therapy - barriers to effective treatment. *Adv Drug Deliv Rev*. 2014. 85: 24-43
13. Singh D, Ravi A, Kane K, Schmalbach T, Hava DL. The pharmacokinetics, pharmacodynamics and tolerability of PUR0200, a novel tiotropium formulation, in chronic obstructive pulmonary disease. *Br J Clin Pharmacol*. 2018;84(9):2097-2105.
14. Perry J, Trautman B, Takher-Smith J, et al. Particle size and gastrointestinal absorption influence tiotropium pharmacokinetics: a pilot bioequivalence study of PUR0200 and Spiriva HandiHaler. *Br J Clin Pharmacol*. 2019;85(3):580-589.
15. Asthma, G.I.f., Global Strategy for Asthma Management and Prevention (2017). 2017.
16. Buckeridge C, Duvvuri S, Denney WS. Simple, automatic noncompartmental analysis: the PKNCA R package. *J Pharmacokinet Pharmacodyn*. 2015;42(1):11-107.
17. Borman AM et al. MIC distributions *and Evaluation of Fungicidal Activity for Amphotericin B, Itraconazole, Voriconazole, Posaconazole and Caspofungin and 20 Species of Pathogenic Filamentous Fungi Determined Using the CLSI Broth Microdilution Method. J Fungi (Basel)*. 2017; 3(2). 27-41
18. Lepak AJ, Andes DR. Antifungal pharmacokinetics and pharmacodynamics. *Cold Spring Harb Perspect Med*. 2015;5(5):1-23. a019653
19. Geller DE, Weers J, Heuerding S. Development of an inhaled dry-powder formulation of tobramycin using PulmoSphere technology. *J Aerosol Med Pulm Drug Deliv*. 2011;24(4):175-182.
20. Hamed K, Debonnett L. Tobramycin inhalation powder for the treatment of pulmonary Pseudomonas aeruginosa infection in patients with cystic fibrosis: a review based on clinical evidence. *Ther Adv Respir Dis*. 2017;11(5):193-209.
21. Skyler JS, Jovanovic L, Klioze S, Reis J, Duggan W, Inhaled Human Insulin Type 1 Diabetes Study Group. Two-year safety and efficacy of inhaled human insulin (Exubera) in adult patients with type 1 diabetes. *Diabetes Care*. 2007;30(3):579-585.

22. SPORANOX® (itraconazole) Oral Solution. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2009/020657s011s018s019s021lbl.pdf.
23. Groll AH, Townsend R, Desai A, et al. Drug-drug interactions between triazole antifungal agents used to treat invasive aspergillosis and immunosuppressants metabolized by cytochrome P450 3A4. *Transpl Infect Dis*. 2017;19(5). 1-11
24. Hope WW, Billaud EM, Lestner J, Denning DW. Therapeutic drug monitoring for triazoles. *Curr Opin Infect Dis*. 2008;21(6):580-586.
25. Evans C, Cipolla D, Chesworth T, et al. Equivalence considerations for orally inhaled products for local action-ISAM/IPAC-RS European workshop report. *J Aerosol Med Pulm Drug Deliv*. 2012;25(3):117-139.
26. SmPC SPIRIVA® 18 microgram, inhalation powder, hard capsule. Document last updated on the eMC: 04-Feb-2015, date of revision of the text 01/2015, accessed on 09-Jun-2015 at http://www.medicines.org.uk/emc/medicine/10039:1-10.
27. Conte JE Jr, Conte JE, Golden JA, Kipps J, Mclver M, Zurlinden E. Intrapulmonary pharmacokinetics and pharmacodynamics of itraconazole and 14-hydroxyitraconazole at steady state. *Antimicrob Agents Chemother*. 2004;48(10):3823-3827.
28. Barone JA, Koh JG, Bierman RH, et al. Food interaction and steady-state pharmacokinetics of itraconazole capsules in healthy male volunteers. *Antimicrob Agents Chemother*. 1993;37(4):778-784.
29. Patterson TF, Thompson GR 3rd, Denning DW, et al. Practice guidelines for the diagnosis and Management of Aspergillosis: 2016 update by the Infectious Diseases Society of America. *Clin Infect Dis*. 2016;63(4):e1-e60.
30. Denning DW, van Wye J, Lewiston NJ, Stevens DA. Adjunctive therapy of allergic bronchopulmonary aspergillosis with itraconazole. *Chest*. 1991;100(3):813-819.
31. Conway SP, Etherington C, Peckham DG, Brownlee KG, Whitehead A, Cunliffe H. Pharmacokinetics and safety of itraconazole in patients with cystic fibrosis. *J Antimicrob Chemother*. 2004;53(5):841-847.
32. Prentice AG, Glasmacher A. Making sense of itraconazole pharmacokinetics. *J Antimicrob Chemother*. 2005;56(Suppl 1):i17-i22.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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