Synopsis

Title of Study: A Randomized, Double-Blind, Multicenter, Placebo-Controlled, Phase 2 Study to Evaluate the Safety, Tolerability, and Pharmacokinetics of Itraconazole Administered as a Dry Powder for Inhalation (PUR1900) in Adult Asthmatic Patients With Allergic Bronchopulmonary Aspergillosis

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Study Centers: This was a multicenter study conducted at the following sites where at least 1 subject was screened: the USA (6 sites), United Kingdom (1 site), Poland (3 sites), India (1 site), and Australia (2 sites).

Publications: None

Study Period (Years): 31 Jul 2019 (first subject first visit) to 14 Apr 2020 (last subject last visit)

Drug Development Phase: 2

Background and Rationale:

Pulmatrix, Inc. (Pulmatrix) is developing PUR1900 as a first-in-class, inhaled, antifungal therapeutic for the treatment of ABPA in patients with asthma. PUR1900 contains

itraconazole as the active ingredient, and it is formulated as a dry powder for oral inhalation. The novel iSPERSE™ dry powder delivery technology enables the efficient delivery of high itraconazole concentrations to the pulmonary airways. Oral doses of itraconazole have demonstrated efficacy in treating ABPA (Stevens et al 2000; Wark et al 2003), but relatively low concentrations in the airway, drug-drug interactions, and side effect profile of enteric itraconazole limits its therapeutic efficacy (Chmiel et al 2014; Saito et al 2012). An inhaled formulation of itraconazole may overcome some of these limitations by achieving higher concentrations in the lungs at a lower systemic exposure than enteric therapy, improving efficacy while reducing risk.

Itraconazole is a broad-acting triazole antifungal agent that has been approved for use in Europe since 1989 and in the United States since 1992 as an orally administered capsule. Coadministration of itraconazole with numerous CYP3A4 substrate medications is contraindicated because of life-threatening cardiac dysrhythmias and/or sudden death in some patients. Itraconazole has been associated with rare cases of serious hepatotoxicity, including liver failure and death (Sporanox® Package Insert 2018).

Pulmatrix conducted a Phase 1, 3-part, open-label study to assess the safety, tolerability, and PK of PUR1900 in healthy subjects (single and multiple doses) and separately in subjects with mild to moderate stable asthma in a crossover comparison with an oral solution of itraconazole (Pulmatrix Study 601-0013). Data from healthy normal subjects (Parts 1 and 2) indicated that PUR1900 was safe and well-tolerated for up to 14 days with daily dosing up to 35 mg, and data from subjects with stable asthma indicated that PUR1900 was safe and well-tolerated administered as a single dose of 20 mg while also achieving minimum inhibitory concentrations required to inhibit the growth of 90% of organisms in airway secretions for 24 hours.

This Phase 2 study was designed to further evaluate the safety, tolerability, and PK of PUR1900 in a target population of adults with asthma and ABPA for a period of 28 days at 3 different doses of PUR1900 compared to placebo dry powder inhalation.

Objectives and Endpoints:

Primary Objective and Endpoints

Objective	Endpoints
To evaluate the safety and tolerability of multiple-dose administration of PUR1900 given to adult subjects with asthma and ABPA	• Incidence of TEAEs • Incidence of intraday FEV_1 declines (from predose to postdose) of ≥10%, ≥15%, and ≥20% • Vital sign measurements (respiratory rate, blood pressure, heart rate, oxygen saturation by pulse oximetry, oral or tympanic temperature) • Physical examination findings • Clinical laboratory test results • 12-Lead ECG findings

Secondary Objectives and Endpoints

Objectives	Endpoints
To characterize the PK of multiple doses of inhaled PUR1900 in plasma and sputum	• Pharmacokinetic parameters of itraconazole and hydroxy-itraconazole in plasma, including, but not limited to, C_{max}, T_{max}, AUC, CL/F, and V_z/F • Sputum concentrations of itraconazole and hydroxy-itraconazole on Day 2, on Day 14, and at follow-up
To evaluate the effect of PUR1900 on biomarkers of inflammation (sputum eosinophils and total serum IgE)	• Change from baseline (Day -9 to Day -6) to Day 28 in sputum eosinophil count • Change from baseline (Day -9 to Day -6) to Day 28 in percentage of sputum eosinophils • Change from baseline (Day 1) to Day 28 in IgE serum concentration
To evaluate the effect of PUR1900 on pulmonary function following single- and multiple-dose administration of PUR1900	• Change from baseline (Day 1) to Day 28 in FEV_1 • Change from baseline (Day 1) to Day 28 in FVC, PEF, and FEV_1/FVC
To evaluate the impact of PUR1900 on respiratory symptoms, as measured by the ACQ-6	• Change from baseline (Day 1) to Day 28 in ACQ-6 score
To evaluate the effect of PUR1900 on the	• Change from baseline (Day 1) to the

Objectives	Endpoints
A fumigatus burden in the sputum, as assessed by quantitative PCR and sputum culture	follow-up visit in *A fumigatus* burden in sputum as assessed by quantitative PCR and sputum culture

Exploratory Objectives and Endpoints

Objectives	Endpoints
To evaluate the effect of PUR1900 on IgE- and IgG-specific antibodies to *A fumigatus*	• Change from baseline (Day 1) to Day 28 in the plasma concentration of IgE and IgG antibodies specific to *A fumigatus* antigens
To evaluate the effect of PUR1900 on sputum ECP	• Change from baseline (Day -9 to Day -6) to Day 28 in sputum ECP
To evaluate the effect of PUR1900 on FeNO	• Change from baseline (Day -9 to Day -6) to Day 28 in FeNO
To evaluate the effect of PUR1900 on sputum neutrophils	• Change from baseline (Day -9 to Day -6) to Day 28 in sputum neutrophil count • Change from baseline (Day -9 to Day -6) to Day 28 in percentage of sputum neutrophils

Methodology:

This was a randomized, double-blind, multicenter, placebo-controlled, multiple-arm study.

Following screening and confirmation of eligibility, subjects were to randomly assign (1:1:1:1) to 4 arms and receive 10 mg, 20 mg, or 35 mg of PUR1900 or placebo, administered via dry powder inhalation daily for 28 days.

Subject eligibility for the study was determined within 28 days before the first dose of study drug (Day 1) and was confirmed between 9 and 6 days before dosing and again on Day 1.

During the screening period, subjects remained on a stable asthma medication regimen (including but not limited to oral and/or inhaled GCS, inhaled SABAs and LABAs, and LTRAs) for a minimum of 28 days before the baseline visit (Day 1).

Eligible subjects began daily dosing with study drug (PUR1900 or placebo) on Day 1. Subjects were trained on the use of the dry powder inhaler device before receiving the first

dose of study drug. The first dose was administered at the study site after all baseline assessments had been completed.

Subjects returned to the study site for visits on Days 2, 7, 14, and 28 and were dosed at the study site. The remaining daily doses of study drug were self-administered at home.

The spirometry measurements assessed at the study sites were evaluated by a central reader using equipment and electronic transfer procedures that are standardized across all sites.

An asthma monitoring device, comprising an integrated spirometer and electronic diary, was used by subjects at home both to measure and record the PEF and to record their symptoms and use of bronchodilator rescue medication throughout the study. At each study visit, the investigator reviewed all home-recorded data for each subject and then determined whether the subject could be allowed to continue participation in the study. If a significant decline in pulmonary function, increase in respiratory symptoms, or increase in rescue medication use was detected by the device, the device alerted the subject to contact the study site.

A follow-up visit occurred 7 to 10 days after the last dose of study drug.

Subjects who withdrew from the study before Day 28 were encouraged to return for an early termination visit. The procedures conducted at the early termination visit were same as the Day 28 procedures. Subjects who discontinued study drug before Day 28 were encouraged to remain in the study and complete all study procedures.

Number of Subjects (Planned and Analyzed):

A sample size of 64 randomly assigned subjects was planned.

The sample size was planned to ensure that a reasonable clinical database to assess safety (the primary study objective) would be obtained. A power calculation based on sputum eosinophil count (a major secondary endpoint) was performed to refine the sample size.

Assuming a 1.45 treatment difference on a logarithmic scale for change from baseline to Week 4 in sputum eosinophils, an SD of 1.07, approximately 5% of subjects discontinued treatment, and a 2-sided significance level of 0.10, 64 subjects (16 randomized subjects per arm) would give at least 90% power to detect a statistically significant difference for each pairwise comparison of PUR1900 treatment group versus the placebo group, for comparisons

to which a minimum 1.45 treatment difference applied. If the proportion of planned randomly assigned subjects who discontinued treatment was greater than the assumed value of 5%, then up to 16 additional subjects (accounting for a treatment discontinuation rate of up to 25%) were to be enrolled and randomly assigned, with the goal of having 60 subjects complete the study.

However, the study was terminated early due to the COVID-19 pandemic, and prior to suspension of enrollment, 7 subjects had been randomly assigned in the study with 5 subjects completing the study.

Diagnosis and Main Criteria for Inclusion and Exclusion:

Inclusion criteria

Each subject had to meet all the following criteria to be enrolled in this study:

1. Could provide written informed consent before the performance of any study-specific procedures.
2. Was a male or female ≥18 and <80 years old at the time of signing the informed consent.
3. Had a body mass index of ≥18.0 and <40.0 kg/m^2 at screening.
4. Had a historical diagnosis of asthma, as per the GINA 2018 update.
5. Had a confirmed historical diagnosis of ABPA, as per the Modified ISHAM working group 2013 criteria (including a serum Ig-E >1000 IU/mL at time of diagnosis).
6. Was considered to be in one of the following stages of ABPA: Stage 2 (Response), Stage 4 (Remission), Stage 5a (Treatment-dependent ABPA), or Stage 5b (Glucocorticoid-dependent asthma).
7. Had a serum IgE ≥500 IU/mL during screening (Visit 1 or Visit 2).
8. Could perform a valid, reproducible spirometry test with demonstration of a prebronchodilator FEV_1 ≥50% of predicted normal for age, sex, race, and height at a screening visit.

9. Had a documented stable asthma medication regimen during screening (Day -28 to Day 1); applicable asthma medications could include any or all of the following: SABA, LABA, and LTRA use and inhaled and/or oral GCS.
10. Subjects who were sexually active, male subjects able to father a child, and female subjects of childbearing potential agreed to follow the contraception requirements outlined in the study protocol.
11. Could demonstrate the correct inhalation technique for the use of the delivery device at screening and before dosing on Day 1.
12. Was willing and able to comply with all study procedures and assessments, including scheduled visits, drug dosing plan, study procedures, laboratory tests, and study restrictions.

Exclusion Criteria

A subject who met any of the following criteria were excluded from the study:

1. Had used any anti-IgE (eg, Xolair® [omalizumab]) or anti-IL-5 biologics (eg, Cinqair® [reslizumab], Nucala® [mepolizumab], or Fasenra® [benralizumab]) in the 6 months before first dose of study drug.
2. Was a female of childbearing potential who was pregnant or lactating or who planned to become pregnant during the study (all female subjects must have had a negative pregnancy test at screening and predose on Day 1). A woman was considered to be of childbearing potential unless she was either permanently sterile (hysterectomy, bilateral salpingectomy, bilateral oophorectomy, bilateral tubal occlusion/ligation) or postmenopausal (had no menses for 12 months without an alternative medical cause).
3. Was taking or had taken any prescribed or over-the-counter drug that was a CYP3A4 inhibitor or substrate in the 14 days (or 5 half-lives, whichever was longer) before first dose of study drug and for the duration of the study (exclusion also applied to the consumption of whole fruit or juices of grapefruit and Seville or pomelo oranges).
4. Was taking or had taken any herbal remedies or CYP3A4 inducers in the 28 days before first dose of study drug.
5. Had used any systemic azole antifungal agent in the 3 months before first dose of study drug.

6. Had a history of life-threatening asthma within the last 5 years, defined as an asthma episode that required intubation and/or was associated with hypercapnia, respiratory arrest, and/or hypoxic seizures.
7. Had an occurrence of asthma or ABPA exacerbations within the 28 days before screening or during the 28-day period before Day 1.
8. Had an occurrence of clinically significant bacterial, viral, or fungal infection that required systemic (oral or intravenous) antibiotics, antivirals, or antifungals within the 28 days before screening. Topical treatments, other than antifungals were allowed.
9. Received any investigational medical product in a clinical research study within the previous 3 months before dosing in this study.
10. Was a study site employee, an immediate family member of a study site employee, or a sponsor employee.
11. Had previously received PUR1900.
12. Had a history of any significant drug or alcohol abuse in the past 2 years before screening, as judged by the investigator.
13. Was using tobacco or inhaled marijuana or had a history of smoking tobacco or marijuana within the last 6 months before screening.
14. Was a current user of e-cigarettes or had used these products within the last 6 months before screening.
15. Had the absence of suitable veins for multiple venipunctures/cannulation as assessed by the investigator or designee at screening.
16. Had evidence or history of clinically significant abnormal serum chemistry, hematology, or urinalysis at screening, as judged by the investigator (particularly elevation of liver enzymes or bilirubin).
17. Had a positive urine test result for drugs of abuse, alcohol, or cotinine at screening (unless, in the opinion of the investigator, this could be explained by the subject's current medications).
18. Had a positive human immunodeficiency virus (HIV; type A and type B) antibody result: a subject who was HIV antibody positive was not excluded if a subsequent CD4 count was ≥200 cells/μL.

19. Had evidence or a history of clinically significant cardiovascular, renal, hepatic, or gastrointestinal disease or neurological or psychiatric disorder, as judged by the investigator.
20. Had evidence or a history of endocrine, immunological, or autoimmune disease that could affect the subject's safety or confound the assessment of study endpoints in the opinion of the investigator.
21. Had a current diagnosis of any chronic airway disease other than asthma, ABPA, or bronchiectasis believed to be related to ABPA, such as chronic obstructive pulmonary disease, pulmonary fibrosis, cystic fibrosis, or Churg-Strauss syndrome. A subject whose predominating clinical disease burden was related to bronchiectasis (eg, a subject with 2 or more infective exacerbations of bronchiectasis in the past 12 months or a subject with chronic colonization with *Pseudomonas aeruginosa*) were excluded.
22. Had evidence of ventricular dysfunction, such as congestive cardiac failure, or a history of congestive cardiac failure. NT-pro BNP was checked at screening only. A subject with a confirmed value of >400 pg/mL was be eligible to participate.
23. Had a 12-lead ECG demonstrating a mean QTcF >450 ms for a male subject or >470 ms for a female subject at screening. A repeat triplicate ECG was allowed if a mean QTcF >450 ms was recorded at Visit 1 or Visit 2.
24. Had a serious adverse reaction or serious hypersensitivity to any of the formulation excipients.
25. Had a history of allergic or hypersensitivity reaction or serious adverse reaction after dosing of itraconazole or other antifungal azoles.
26. Had a major trauma or surgery within the last 28 days before screening.
27. Had a planned or elective surgery, hospitalizations, or participation in other interventional studies any time during the study that could interfere with study logistics or safety.
28. Had donated or had a loss of greater than 400 mL of blood within the 3 months before screening.
29. Had the presence of hoarseness or oropharyngeal candidiasis at screening.
30. Had other social, psychiatric, surgical, or medical conditions or screening laboratory abnormalities that could increase the risk associated with study participation or could interfere with the interpretation of study results and, in the judgment of the investigator, could make the subject inappropriate for entry into the study.

Test Product, Dose and Mode of Administration:

Study drug (PUR1900 or placebo) was administered by oral inhalation, using a dry powder inhaler specific to the study (RS01 Monodose inhaler), once daily for 28 days at approximately the same time each day between 5:00 am and 10:00 am.

PUR1900 was supplied as capsules with 10 mg total powder (5 mg itraconazole plus 5 mg excipients).

Control Product, Dose and Mode of Administration:

Placebo was supplied as capsules with 5.9 mg total powder (excipients only). The placebo capsules were formulated so that the 5.9 mg powder in the placebo capsule matched the appearance of 10 mg of powder in the PUR1900 capsule.

To maintain the blind, every subject received 7 capsules per daily dose, which was a combination of PUR1900 capsules and placebo capsules based on the treatment group assignment of the subject.

PUR1900 capsules and placebo capsules were assembled into child-resistant blister cards that achieved the blinding for the study. The ratio of PUR1900 capsules to placebo capsules in each day's dose varied by treatment group, as follows:

Treatment Group	Daily Dose
10 mg PUR1900	2 PUR1900 capsules and 5 placebo capsules
20 mg PUR1900	4 PUR1900 capsules and 3 placebo capsules
35 mg PUR1900	7 PUR1900 capsules and 0 placebo capsules
Placebo	0 PUR1900 capsules and 7 placebo capsules

Duration of Treatment:

The duration of treatment was 28 days for each subject. The duration of the study was defined for each subject as the date on which signed written informed consent was provided through the last follow-up visit, and it comprised 8 study visits within approximately 9 weeks.

Estimands and Intercurrent Events:

Estimands and intercurrent events were not planned for the study.

Statistical Methods:

Due to the early termination of the study, a limited number of summary tables and listings were generated.

The screened set included of all subjects who signed an informed consent form. The randomized set consisted of all subjects randomly assigned to receive double-blind study treatment regardless of whether or not they received a dose of the study drug. The PK set included all subjects who received at least one dose of active PUR1900 with sufficient concentration data to support the accurate estimation of at least 1 PK parameter (excluding the single-point parameters of C_{max}, T_{max}, and C_{trough}). Subjects in this population were used in all PK summaries and all PK analyses and comparisons. Safety data were presented using the safety set, which consisted of all randomized subjects who took at least 1 dose of study drug, with subjects analyzed according to the treatment group for the study drug that they actually received.

The demographic characteristics consisting of age (years), gender, race, and ethnicity and baseline characteristics were presented in a listing using unit as collected in the CRF for height and weight for the Screened Set.

Prior and concomitants medications, drug accountability, and exposure dates were listed using the Safety Set.

Safety:

Adverse events were coded using the MedDRA Version 21.0. The data for AEs, spirometry, ECG, vital signs, and laboratory results (hematology, serum chemistry, and urinalysis) were presented in the listings by subject.

These data were presented using Safety Set.

Pharmacokinetics:

Plasma concentration data of itraconazole and hydroxy-itraconazole were summarized by time point for each treatment using the following descriptive statistics: number of subjects, arithmetic mean, arithmetic SD, arithmetic CV, geometric mean, geometric CV, median, minimum, and maximum. Mean plasma concentration-versus-time profiles were presented using nominal time, and individual plasma concentration-versus- time profiles were presented using actual time on both linear and semilogarithmic scales.

The individual plasma PK parameters for itraconazole and hydroxy-itraconazole were to be presented in data listings and summarized by treatment using the following descriptive statistics: n, mean, SD, CV, median, minimum, and maximum. Geometric means and geometric CV were included for AUC and C_{max} values.

An assessment of plasma steady state was presented using a graphical assessment of mean trough concentrations on Days 7, 14, and 28 for each group.

Pharmacodynamics

Sputum eosinophils, total IgE serum concentration, and *Aspergillus fumigatus* burden in sputum (assessed by sputum culture and PCR) were listed using the Screened Set.

Summary of Results:

The study was terminated early due to the COVID-19 pandemic. Pulmatrix was monitoring the pandemic over several months, with particular attention to the trends in case rates in areas where the study sites were located. The analysis of these trends led to conclusion that it was unlikely that conditions would allow for a safe and uninterrupted resumption of the study within a reasonable timeframe. Therefore, Pulmatrix decided to terminate the study on 15 Jul 2020.

Prior to suspension of enrollment, 7 subjects had been randomly assigned in the study with 5 subjects completing the study. The subjects were enrolled from 1 site each in the USA, UK, Poland, India, and Australia. Subject disposition is summarized in the Table 1.

One subject in the PUR1900 35 mg group was withdrawn early from the study due to protocol violation and 1 subject in the placebo group withdrew consent (Listing 16.2.3.1). For more details please refer to the individual subject narratives.

There were no deaths, related SAEs, or AEs leading to study drug discontinuation during the study.

Table 1 Subject Disposition (Randomized Set)

	PUR1900 10 mg (N=2)	PUR1900 20 mg (N=2)	PUR1900 35 mg (N=1)	PUR1900 All doses (N=5)	Placebo (N=2)	Total (N=7)
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Total number of subjects randomized	2	2	1	5	2	7
Received at least one dose	2 (100)	2 (100)	1 (100)	5 (100)	2 (100)	7 (100)
Completed treatment period	2 (100)	2 (100)	0	4 (80.0)	1 (50.0)	5 (71.4)
Discontinued study treatment	0	0	1 (100)	1 (20.0)	1 (50.0)	2 (28.6)
Completed study	2 (100)	2 (100)	0	4 (80.0)	1 (50.0)	5 (71.4)
Early termination	0	0	1 (100)	1 (20.0)	1 (50.0)	2 (28.6)

Notes: N = Number of subjects in randomized set population, n = Number of observations.
Percentages were based on the number of subjects randomized.
Source Data: Table 14.1.2.1

The PUR1900 plasma concentration data are presented in Table 14.2.1.1 and Listing 16.2.6.3. The PUR1900 plasma PK parameters are summarized by treatment on Day 1 and 28 in Table 2 and in Table 14.2.1.2 and Table 14.2.1.3, respectively.

Table 2 Summary of Plasma Pharmacokinetic Parameters of Itraconazole and Hydroxy-itraconazole — Day 1 and Day 28 (Pharmacokinetic Set)

Day Statistics	AUC_{0-t} (h·ng/mL)			AUC_{0-tau} (h·ng/mL)			C_{max} (ng/mL)			T_{max} (h)		
	PUR1900 10 mg	PUR1900 20 mg	PUR1900 35 mg	PUR1900 10 mg	PUR1900 20 mg	PUR1900 35 mg	PUR1900 10 mg	PUR1900 20 mg	PUR1900 35 mg	PUR1900 10 mg	PUR1900 20 mg	PUR1900 35 mg
Day 1												
N	2	2	1	2	2	1	2	2	1	2	2	1
n	2	2	1	2	2	1	2	2	1	2	2	1
Geometric mean (CV%)	30.1 (64.4)	45.4 (61.0)	47.5 (-)	29.8 (65.8)	44.6 (58.0)	47.5 (-)	1.57 (53.3)	3.03 (44.8)	2.44 (-)	- (-)	- (-)	- (-)
Median	32.7	49.0	47.5	32.6	47.9	47.5	1.67	3.17	2.44	14.80	4.03	6.00
Minimum	19.8	30.5	47.5	19.5	30.5	47.5	1.10	2.24	2.44	6.05	3.97	6.00
maximum	45.6	67.5	47.5	45.6	65.3	47.5	2.23	4.10	2.44	23.55	4.08	6.00
Day 28												
N	2	2	-	2	2	-	2	2	-	2	2	-
n	2	2	-	2	2	-	2	2	-	2	2	-
Geometric mean (CV%)	401 (80.5)	647 (61.0)	- (-)	108 (88.2)	158 (74.2)	- (-)	5.11 (92.6)	9.17 (84.4)	- (-)	- (-)	- (-)	- (-)
Median	452	699	-	124	175	-	5.93	10.4	-	6.01	3.05	-
Minimum	243	435	-	63.3	98.7	-	2.93	5.46	-	4.00	2.10	-
maximum	661	963	-	185	252	-	8.92	15.4	-	8.02	4.00	-

Notes: N = Number of subjects in pharmacokinetic population, n = Number of observations.
On Day 28, AUC_{0-t} represents the area under the curve from time zero to follow-up (approximately 168 hours post-dose).
There is no data for the PUR1900 35 mg treatment on Day 28
Source Data: Table 14.2.1.2 and Table 14.2.1.3.

The data for total serum IgE concentration and serum IgG and IgE *A fumigatus*-specific antibodies concentration and data for eosinophil, ECP, neutrophil in sputum are presented in the Listing 16.2.6.1 and Listing 16.2.6.2, respectively. The spirometry data are presented in the Listing 16.2.4.2. Due to the small number of subjects in the dosing groups, no conclusions can be drawn from these data.

The data for *A fumigatus* burden in sputum is presented in Listing 16.2.6.2.1. Sputum samples were collected from each subject at baseline, on Day 28, and at the follow- up visit. For each sample, 3 separate assessment were made where possible. Specimens were processed by HVC, using undiluted sputum on Sabouraud agar at 30oC for up to 14 days according to the methods described by Vergidis et al. A separate specimen was tested by quantitative real-time PCR for the isolation of fungal nucleic acids present in the samples and analyzed for the presence of *A fumigatus* DNA using the quantitative ELITech Aspergillus spp. ELITe MGB qPCR kit. Antifungal susceptibility testing was performed by the EUCAST standard (Arendrup et al). For the rt-PCR, interpretation of DNA copy numbers is as follows;

<300 DNA copies = Negative, no Aspergillus DNA detected.
301 to 459 DNA copies = Weak Positive, some Aspergillus DNA detected.
>460 DNA copies = Strong Positive, Aspergillus DNA detected.

For this study, 7 subjects were enrolled in total. One subject was withdrawn early when he was found to not meet several inclusion criteria. Two subjects completed all doses but were both unable at any time point to produce sufficient sputum for analysis. Three subjects produced a sputum sample at baseline, which returned negative for *A fumigatus*, from which no colonies could be cultured or from which only colonies other than *A fumigatus* could be cultured. These included a variety of moulds across subject and sample including Penicillium, *A. niger,* Epicoccum, *Fusarium chlamydosporum*, *Fusarium equiseti*, *Fusarium nygamai*, *Polyporus arcularius*, *Talaromyces macrosporus* and *Ceriporia lacerate*. Accordingly, only 1 subject consistently produced samples across the study that consistently included *A fumigatus*, either from culture of via rt-PCR analysis.

Subject 203002 had insufficient sample for culture and susceptibility at baseline and at the follow-up visit. However, the rt-PCR data from the baseline sample showed a DNA copies value of 3353 indicating a strong positive for aspergillus colonization. This individual completed 28 days of dosing with 10 mg PUR1900, at which time sputum culture was negative for *A fumigatus* but included 3 strains of Penicillium. From this Day 28 sample,

rt-PCR data showed a DNA copies value of 197 indicating a negative result for aspergillus. Susceptibility testing on the 3 penicillium isolates showed susceptibility in isolates 1 and 2 but MICs above 8 mg/L for isolate #3 for itraconazole and voriconazole. From the post study follow-up sputum sample, there was again insufficient sample for culture or susceptibility, but the rt-PCR data returned a DNA copies value of 312, just above the lower limit for weak positive. Combined, these data suggest that treatment with 10 mg PUR1900 for 28 days resulted in a substantial reduction in aspergillus as determined by rt-PCR analysis. There was insufficient data for culture and susceptibility at 2 of the 3 timepoints to make an interpretation on those endpoints.

Overall, there were insufficient sputum samples produced across the study, or insufficient samples showing *A fumigatus* colonization to allow for any reliable interpretation of culture or susceptibility data. Results for the 1 subject with a strong rt-PCR positive for *A fumigatus* at baseline with negative or weakly positive thereafter is suggestive of an effect of dosing reducing the aspergillus levels, but no definitive conclusion can be taken from a single subject.

The data for ECG, vital signs, and laboratory results (hematology, serum chemistry, and urinalysis) are presented in Listings 16.2.8.1, Listing 16.2.8.2, and Listing 16.2.8.3, respectively. No significant changes or abnormalities were observed in these measurements during study participation. However, due to the small number of subjects in the dosing groups, no conclusions can be drawn from these data.

Subject Narratives

Data listings: 16.2.1.1, 16.2.2.1, 16.2.3.1, 16.2.4.1, 16.2.4.3, 16.2.5.1, 16.2.7.1, 16.2.9.1.

The brief narrative for all 7 randomly assigned subjects are presented below.

Subject ID: 111001

A 75-year-old White male subject was enrolled in the study on 07 Aug 2019. His height was 70 in., weight was 183.4 lb, and BMI was 26.30 kg/m^2 at baseline.

The subject's concomitant medications and concurrent medical conditions included macrogol/propylene glycol for dry eyes; montelukast for seasonal allergies; omeprazole for GERD; fluticasone/salmeterol and salbutamol for asthma; metoprolol and losartan for hypertension; acetylsalicylic acid for cardiac prophylaxis; atorvastatin for hyperlipidemia; furosemide for peripheral edema; and potassium chloride for hypertension and peripheral edema.

The subject was randomly assigned to the PUR1900 35 mg arm on 30 Aug 2019.

On Day –10 (01 Sep 2019), the subject experienced non-serious AEs of mild eye pruritus (bilateral eye itching [RT]) and mild rhinorrhoea. Both the AEs were resolved without any additional treatment on Day –1 (10 Sep 2019). As assessed by the investigator, the events were not respiratory events of clinical concern and were not attributed to the inhaler device. The investigator assessed the AEs eye pruritus and rhinorrhoea to be not related to study drug.

On the next day (Day 1; 11 Sep 2019), the subject received the first dose of study drug. A site monitoring visit that occurred during the first week of dosing revealed that the subject did not meet inclusion criteria # 4, 5, 6, and 7. These findings were considered to be important protocol deviations (Listing 16.2.2.1). Therefore, the Sponsor requested immediate study drug discontinuation, which occurred on Day 7 (17 Sep 2019). The subject was subsequently withdrawn from the study on Day 14 (24 Sep 2019).

Subject ID: 203001

A 74-year-old White male subject was enrolled in the study on 09 Oct 2019. His height was 176 cm, weight was 78.1 kg, and BMI was 25.20 kg/m^2 at baseline.

The subject's concomitant medications and concurrent medical conditions included rosuvastatin for hyperlipidemia; salbutamol, beclometasone/formoterol, prednisolone, and montelukast for asthma; alendronic acid for prevention of osteoporosis; and sodium chloride and carbocisteine for bronchiectasis.

The subject was randomly assigned to the PUR1900 10 mg arm on 30 Oct 2019 and received first dose of study drug on Day 1 (12 Nov 2019).

On Day 17 (28 Nov 2019), the subject experienced non-serious AEs of moderate fatigue and mild dyspnoea. No action was taken with study drug due to either event. Both the AEs were resolved without any additional treatment on Day 20 (01 Dec 2019). As assessed by the investigator, the events were not respiratory events of clinical concern and were not attributed to the inhaler device. The investigator assessed the AEs fatigue and dyspnoea to be not related to study drug.

The subject received the last dose of study drug on Day 28 (09 Dec 2019) and completed the study on Day 37 (18 Dec 2019).

Subject ID: 203002

A 54-year-old White male subject was enrolled in the study on 22 Oct 2019. His height was 182.1 cm, weight was 75.5 kg, and BMI was 22.80 kg/m^2 at baseline.

The subject's concomitant medications and concurrent medical condition included salbutamol, fluticasone, and beclometasone/formoterol for asthma.

The subject was randomly assigned to the PUR1900 10 mg arm on 11 Nov 2019 and received first dose of study drug on Day 1 (18 Nov 2019).

On 06 Dec 2019, he received influenza vaccine.

No AEs were reported for the subject.

The subject received the last dose of study drug on Day 29 (16 Dec 2019) and completed the study on Day 36 (23 Dec 2019).

Subject ID: 301001

A 72-year-old White female subject was enrolled in the study on 11 Dec 2019. Her height was 153 cm, weight was 81.5 kg, and BMI was 34.80 kg/m^2 at baseline.

The subject's concomitant medications and concurrent medical conditions included amlodipine, bisoprolol, hydrochlorothiazide, and ramipril for hypertension; and budesonide/formoterol, prednisone, and salbutamol for asthma.

The subject was randomly assigned to the PUR1900 20 mg arm on 02 Jan 2020 and received first dose of study drug on Day 1 (08 Jan 2020).

No AEs were reported for the subject.

The subject received the last dose of study drug on Day 28 (04 Feb 2020) and completed the study on Day 36 (12 Feb 2020). During a remote monitoring visit on 17 Apr 2020, it was noted that the subject met exclusion criterion #3. Specifically, it was discovered that the subject was receiving amlodipine, and systemic concentrations of this drug have been known to increase when co-administered with itraconazole. However, observations of hypotension, edema, or other signs of amlodipine toxicity were not recorded. This important protocol deviation was discovered after the subject completed study participation, which coincided with the study being paused (and eventually terminated) due to the COVID-19 pandemic. Thus, no action was taken.

Subject ID: 403001

A 29-year-old Asian male subject was enrolled in the study on 05 Feb 2020. His height was 162 cm, weight was 48.8 kg, and BMI was 18.60 kg/m^2 at baseline.

The subject's concomitant medications and concurrent medical condition included desloratadine, montelukast, budesonide, and formoterol for asthma.

The subject was randomly assigned to the Placebo arm on 25 Feb 2020 and received first dose of study drug on Day 1 (04 Mar 2020).

On the next day (Day 2, 05 Mar 2020), the subject discontinued study drug and on Day 6 (09 Mar 2020) he withdrew himself from the study stating that he would not be able to commit to future study days.

No adverse events were reported for the subject.

Subject ID: 501001:

A 59-year-old White female subject was enrolled in the study on 18 Oct 2019. Her height was 163 cm, weight was 74.9 kg, and BMI was 28.20 kg/m^2 at baseline.

The subject's concomitant medication and concurrent medical condition included budesonide/formoterol for asthma.

The subject was randomly assigned to the PUR1900 20 mg arm on 12 Nov 2019 and received first dose of study drug on Day 1 (21 Nov 2019).

On Day 6 (26 Nov 2019), the subject fell and was taken to the emergency department. Subsequently, SAEs of acetabulum fracture (fracture of right acetabulum [RT]) and ulna fracture (fracture to proximal left ulna [RT]) were reported based on the results of a computerized tomography scan of the pelvis and an X-ray scan of the left elbow, respectively. Both these SAEs were severe. The subject also had a non-serious AE of mild laceration (laceration overlying anterior aspect of knee joint [RT]) on the same day. The subject was hospitalized for surgery on Day 7 (27 Nov 2019).

The study drug was interrupted on 27 Nov 2019 due to these SAEs and was resumed on 28 Nov 2019.

The subject was treated with cefazolin, diphtheria vaccine toxoid/tetanus vaccine toxoid, fentanyl, ondansetron, paracetamol, oxycodone, cefalexin, pregabalin, docusate sodium, Microlax, macrogol/potassium chloride/sodium bicarbonate/sodium chloride, and docusate sodium/sennoside A+B.

On Day 12 (02 Dec 2019), acetabulum fracture and ulna fracture were resolved with sequelae; the laceration was also resolved on the same day and the subject was discharged from hospital. The investigator assessed these 3 AEs to be not related to study drug.

The subject received the last dose of study drug on Day 28 (18 Dec 2019) and completed the study on Day 40 (30 Dec 2019).

Subject ID: 501002

A 27-year-old Asian female subject was enrolled in the study on 04 Feb 2020. Her height was 163 cm, weight was 54.4 kg, and BMI was 20.50 kg/m^2 at baseline.

The subject's concomitant medications and concurrent medical conditions included fluticasone/formoterol, salbutamol, and tiotropium for asthma; calcium citrate/colecalciferol/lithothamnium calcareum/menaquinone-7 and zinc as supplements.

The subject was randomly assigned to the Placebo arm on 26 Feb 2020 and received the first dose of study drug on Day 1 (10 Mar 2020).

On 26 Mar 2020, she received the annual influenza vaccine.

No AEs were reported for the subject.

The subject received the last dose of study drug on Day 29 (07 Apr 2020) and completed the study on Day 36 (14 Apr 2020).

Conclusions: The study was terminated early due to COVID-19 pandemic. Prior to suspension of enrollment, 7 subjects had been randomly assigned in the study with 5 subjects completing the study. PUR1900 at the dose of 10 mg, 20 mg, and 35 mg was found to be safe and well-tolerated by the subjects enrolled in the study.

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