SYSTEMIC AND PULMONARY EXPOSURE OF PUR1900 FOLLOWING INHALATION DOSING IN RATS AND DOGS OVER 28 DAYS

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Introduction

Pulmonary fungal infections in Cystic Fibrosis are likely underdiagnosed[1], ranging from allergic bronchopulmonary aspergillosis to invasive aspergillosis that can lead to complications including hemoptysis and can be a significant source of morbidity and mortality. Oral triazole treatment is commonly prescribed, yet historically, oral bioavailability and achieved lung concentrations are variable and often subtherapeutic[2]. In addition, triazoles like itraconazole have extensive systemic toxicities and an multiple drug-drug interaction (DDI) liabilities, including with ivacaftor[3]. Pulmatrix have developed Pulmazole™ (PUR1900), an inhaled dry powder formulation of itraconazole. Over short term dosing studies, this formulation has demonstrated the potential for improved local delivery and efficacy, reduced variability in lung exposure and lower DDI propensity.

PUR1900 is formulated using our proprietary particle engineered dry powder platform called iSPERSE (Figure 1) and is engineered to have a small aerosol particle size for efficient pulmonary delivery and is intended to be delivered using a capsule based dry powder inhaler. PUR1900 has a mass median aerosol diameters (MMAD) of ~3mm and high fine particle doses (FPD; % of the nominal dose < 5mm), which should result in more than 50% of the nominal dose reaching the lungs with reduced throat deposition. Notably, the aerosol target range of PUR1900 is similar to that of *Aspergillus* conidia, which, in theory, should result in PUR1900 delivery to lung sites where aspergillus spores also deposit.

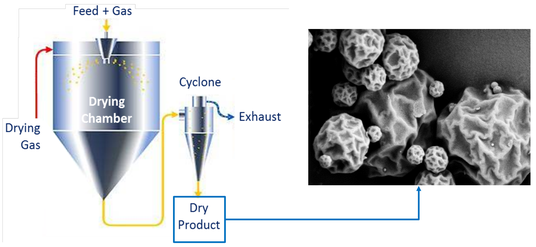

Figure 1. iSPERSE particle engineering. iSPERSE utilizes the combination of particle engineering by spray drying and novel excipient combinations to create homogenous particles for inhalation. iSPERSE particles of different physicochemical properties and morphologies can be achieved through changes in process and excipient selection and allow for the delivery of high drug loads directly to the lung. iSPERSE engineered particles have a consistent and reproducible normal particle size distribution, with a Mass Median Aerodynamic Diameter (MMAD) that is both ideal for distribution throughout the lung, with minimal throat deposition, but is also very similar in size to *Aspergillus* conidia and therefore, in principle, should favor deposition at the site of infection.

Aims

- To evaluate the systemic pharmacokinetics and lung concentrations of itraconazole when PUR1900 is delivered to the lungs of rats and dogs
- To assess systemic and lung accumulation with repeat dosing and determine lung:plasma exposure ratio

Methods

28-Day Pharmacokinetic Study

Sprague-Dawley rats were dosed daily *via* nose-only inhalation exposure at a target delivered inhaled doses of 5, 20 and 44mg/kg/day PUR1900 for 28 consecutive days. Exposure was performed using a rotating brush aerosol generator and directed flow inhalation system Additional groups of rats were dosed with placebo or air controls for 28 consecutive days. Achieved delivered doses are shown below in Table 1.

Table 1. Group mean target and achieved inhaled doses in rats.

Group	Treatment	Target Delivered Dose (mg/kg/day)	Achieved Delivered Dose (mg/kg/day)	Mass Median Aerodynamic Diameter (µm)
1	Air Control	0	0	-
2	Placebo Control	0	0	2.3
3	PUR1900	5	5.8	2.1
4	PUR1900	20	22	1.9
5	PUR1900	44	49	1.8

Purebred Beagle dogs were dosed daily *via* oronasal inhalation using a facemask at a target delivered inhaled doses of 5, 10 and 20mg/kg/day PUR1900 for 28 consecutive days. Exposure was performed using a rotating brush aerosol generator and directed flow inhalation system. Dogs were lightly restrained with a harness system and trained to accept the facemask Additional groups of dogs were dosed with placebo or air controls for 28 consecutive days. Achieved delivered doses are shown below in Table 2.

Table 2. Group mean target and achieved inhaled doses in dogs.

Group	Treatment	Target Delivered Dose (mg/kg/day)	Achieved Delivered Dose (mg/kg/day)	Mass Median Aerodynamic Diameter (µm)
1	Air Control	0	0	-
2	Placebo Control	0	0	2.7
3	PUR1900	5	4.9	2.3
4	PUR1900	10	10	2.4
5	PUR1900	20	22	2.3

In both studies, blood samples for determination of plasma concentrations of itraconazole, were collected from dosed animals on Days 1, 14 and 28 immediately after the end of dosing (IAD - within 5 minutes) and 2, 4, 8 and 24 hours after dose.

In addition, lung samples were collected from rats on Day 1 within 1 hour after dose and 24 hours after dose and on Days 14 and 28 at 24 hours after dose. Lung samples were collected from dogs at 24 hours after the last dose on Day 28 only.

Plasma and lung samples were analyzed by by LC-MS/MS. Pharmacokinetic analysis was performed with WinNonlin PhoenixTM software version 6.3 using individual animal data and non-compartmental analysis

Results

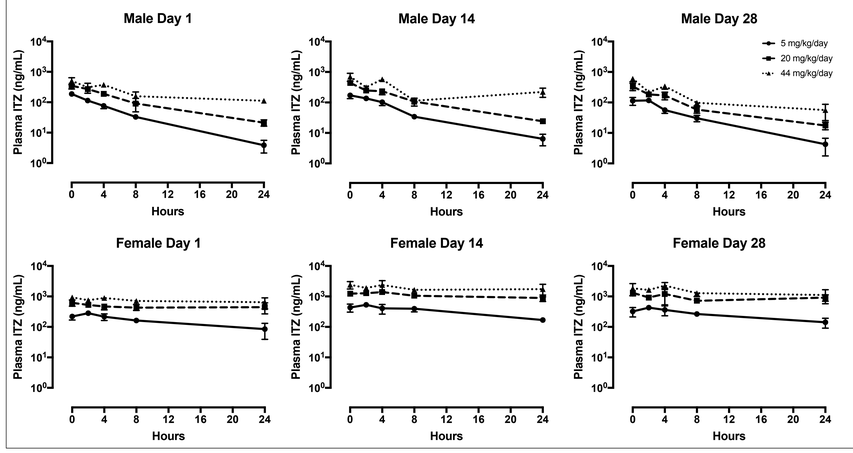

Figure 2. Systemic pharmacokinetic profile in rats. The C_{max} and $AUC_{0\text{-}25h}$ of systemic exposure increased with increasing dose in a less than dose proportionate manner on each day. The dose-adjusted C_{max} and $AUC_{0\text{-}25h}$ of systemic exposure of female rats were approximately 2.7-fold and 6.3 fold higher respectively overall than those in males and tended to be more marked on Day 14 and Day 28. After repeated administration (Day 14 and Day 28) the C_{max} and $AUC_{0\text{-}25h}$ of systemic exposure of male rats to itraconazole were generally similar to those values after a single dose (Day 1), however, systemic exposure of female rats to itraconazole on Day 14 and Day 28 was higher than that after a single dose (Day 1). The accumulation ratios, based on $AUC_{0\text{-}25h}$ values corrected for the achieved doses on each sampling day were generally close to or less than one in males (0.87 to 1.5) and greater than one in females (2.1 to 2.9-fold higher), indicating that accumulation of itraconazole occurred in females after repeated inhalation exposure.

Figure 3. Systemic pharmacokinetic profile in dogs. The C_{max} and $AUC_{0\text{-}25h}$ of systemic exposure of dogs to itraconazole, increased approximately dose proportionally on Day 1, Day 13 and Day 28 with no sex differences. After repeated administration by inhalation the C_{max} and $AUC_{0\text{-}25h}$ were higher than values after a single dose. The accumulation ratios, based on individual animal $AUC_{0\text{-}25h}$ values corrected for the estimated group mean doses on each sampling day, ranged from 1.5- to 5.2-fold indicating accumulation at all doses

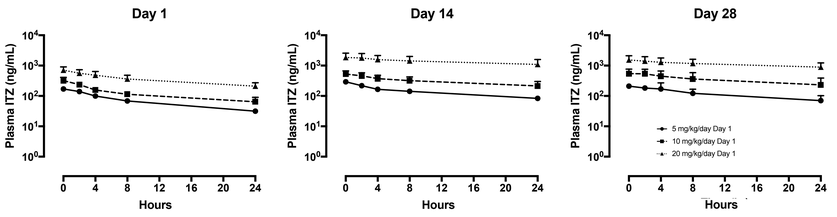

Figure 4. Lung Exposure in rats. The lung concentrations of itraconazole increased approximately dose-proportionately with increasing dose. Dose-adjusted concentrations in females were generally similar to those in males at 1 hour after Day 1 dose, but higher in females at 24 hours after dose on Days 1, 14 and 28, ranging between 1.6 and 5.4-fold higher. After repeated administration by inhalation, accumulation ratios in the lung, based on the concentrations at 24 hours post dose ranged from 4.1- to 24.3-fold in males and 2.1- to 15.5-fold in females and increased with increasing dose in both sexes through Day 28. Lung:Plasma ratios are shown in Table 3.

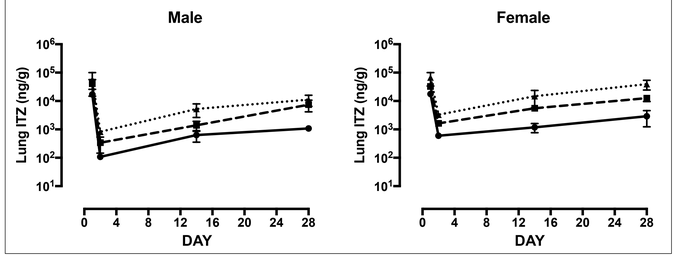

Table 3. Lung:Plasma ratios at equivalent time points in rats

Time point	Dose (mg/kg/day)	Lung:Plasma ratio Males	Lung:Plasma ratio Females
Day 1 IAD	5	91	80
Day 1 Trough	5	28	7
Day 14 Trough	5	98	7
Day 28 Trough	5	254	21
Day 1 IAD	20	119	53
Day 1 Trough	20	16	4
Day 14 Trough	20	58	6
Day 28 Trough	20	422	14
Day 1 IAD	44	113	72
Day 1 Trough	44	7	5
Day 14 Trough	44	24	8
Day 28 Trough	44	200	35

Lung Exposure in dogs.
The lung concentrations in dogs were measured at 24 hours after the last dose and increased with increasing dose in a greater than dose proportionate increment. Day 28 Trough comparisons for the dog ranged from 46- to 108-fold higher levels in the lungs of dogs

Conclusions

- Inhaled PUR1900 provides high and consistent lung exposure over 28-days of dosing with low systemic exposure and high lung:plasma ratios in both species
- Rats show sex differences in exposure and accumulation potential, though these appear to be species specific as dogs show no sex differences
- These data indicate that Pulmazole™ shows potential as an inhaled therapy for pulmonary fungal infection.

References

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