Inhalation improves pirfenidone animal efficacy through superior pulmonary and vascular pharmacokinetics

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INTRODUCTION

IPF is a fatal orphan lung disease characterized by progressive scarring, reduced exercise capacity and death from respiratory failure or co-morbidities. With a 3-5 year survival period, IPF has more deaths per year than breast cancer and only lung cancer has a worse 5 year prognosis. World-wide, only oral pirfenidone is approved to treat IPF. There are no US-approved treatments leaving lung transplant as the only option to extend life.

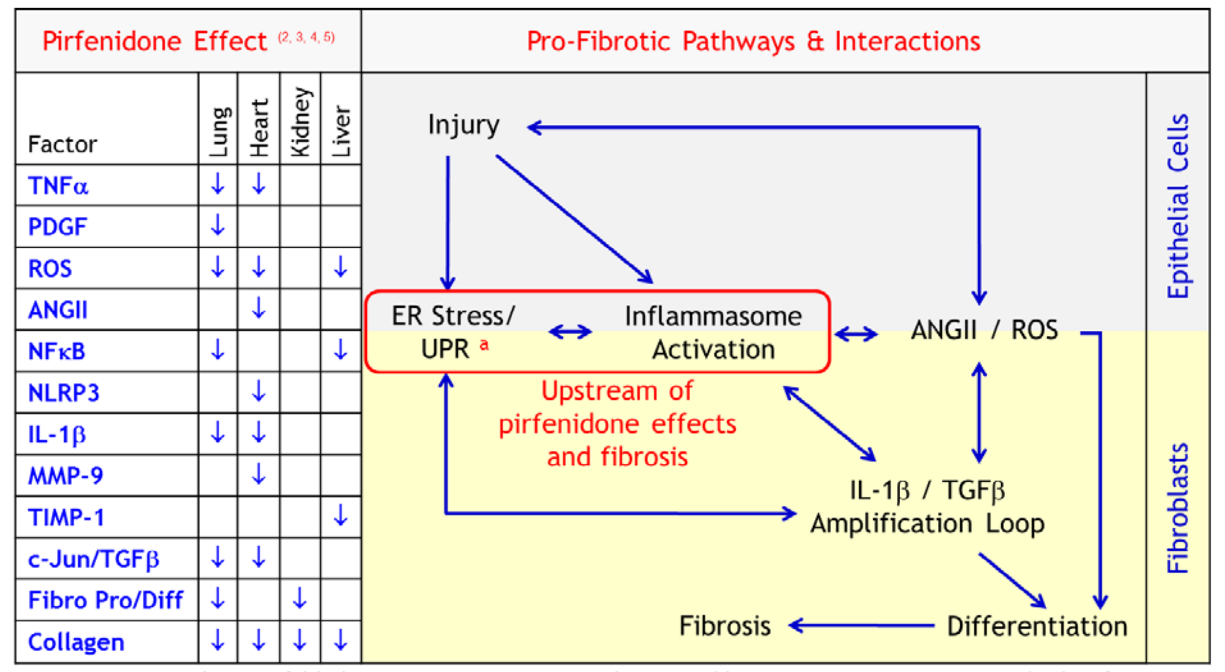
Although oral pirfenidone has shown clinical promise to slow IPF disease progression, as a low potency drug a very large oral dose is required to achieve efficacious lung levels. While the EU-approved oral dose is large (801 mg TID), resulting lung levels are well-below the pirfenidone IC_{50} and associated blood levels remain unsafe and poorly tolerated (preventing dose escalation for additional efficacy). Complicating matters, dose-absorbing food, first-pass metabolism and safety-driven dose-reduction/stoppage protocols(1) further reduce lung dose and interrupt desired maintenance therapy.

To address the above shortcomings and maximize pirfenidone's therapeutic potential, Genoa is developing aerosol pirfenidone (GP-101) for nebulization and inhaled lung administration. By this approach, small inhaled doses deliver oral-superior animal lung levels with reduced systemic exposure. Using this method, we propose that inhaled GP-101 will serve as a safe and well-tolerated oral-alternative and improved-effect IPF therapy.

KEY PHARMACOLOGY QUESTIONS

1. Can inhalation deliver large lung dose with low blood levels?
   - Small inhaled doses deliver a high lung Cmax with low blood levels
   - Relatively short-duration of lung exposure
2. Is short-duration lung exposure sufficient for activity?
   - Only short-duration exposure required for maximum activity
   - Promising to employ inhaled Cmax advantage in IPF therapy
3. Are small inhaled doses effective in animals?
   - Effective in two pro-fibrotic animal models
   - Inhaled efficacy stronger than larger oral dose levels

OBSERVED PIRFENIDONE EFFECTS

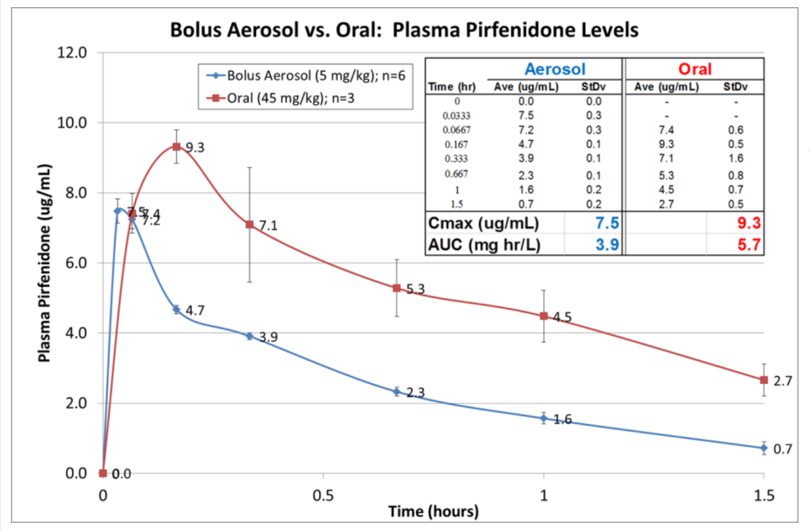


Pirfenidone Effect (2, 3, 4, 5) Factor	Lung	Heart	Kidney	Liver
TNFα	↓	↓		
PDGF	↓			
ROS	↓	↓		↓
ANGII		↓		
NFκB	↓			↓
NLRP3		↓		
IL-1β	↓	↓		
MMP-9		↓		
TIMP-1				↓
c-Jun/TGFβ	↓	↓		
Fibro Pro/Diff	↓		↓	
Collagen	↓	↓	↓	↓

a. ER stress stimulates unfolded protein response (UPR). Also caused by: SFTP mutations, ANGII and other factors.

RESULTS

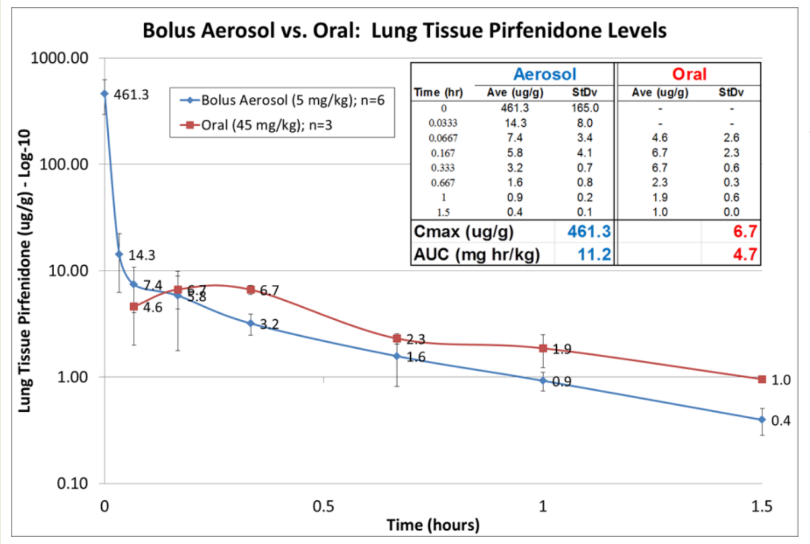
1. Can inhalation deliver large lung dose with low blood levels?



Time (hr)	Aerosol Ave (ug/mL)	Aerosol StDv	Oral Ave (ug/mL)	Oral StDv
0	0.0	0.0	-	-
0.0333	7.5	0.3	-	-
0.0667	7.2	0.3	7.4	0.6
0.167	4.7	0.1	9.3	0.5
0.333	3.9	0.1	7.1	1.6
0.667	2.3	0.1	5.3	0.8
1	1.6	0.2	4.5	0.7
1.5	0.7	0.2	2.7	0.5
Cmax (ug/mL)	7.5		9.3	
AUC (mg hr/L)	3.9		5.7	

Figure 1. Pirfenidone plasma levels following IT and PO administration

- Experiment targeted equivalent blood levels
- IT delivers earlier Tmax. Following Tmax, similar blood PK as oral route



Time (hr)	Aerosol Ave (ug/g)	Aerosol StDv	Oral Ave (ug/g)	Oral StDv
0	461.3	165.0	-	-
0.0333	14.3	8.0	-	-
0.0667	7.4	3.4	4.6	2.6
0.167	5.8	4.1	6.7	2.3
0.333	3.2	0.7	6.7	0.6
0.667	1.6	0.8	2.3	0.3
1	0.9	0.2	1.9	0.6
1.5	0.4	0.1	1.0	0.0
Cmax (ug/g)	461.3		6.7	
AUC (mg hr/kg)	11.2		4.7	

Figure 2. Pirfenidone lung levels following IT and PO administration

- At equivalent blood levels, 9-fold less IT pirfenidone delivers 69-fold greater lung Cmax
- >78-fold increased IT therapeutic index
- Relatively short-duration lung exposure

2. Is short-duration exposure sufficient for activity?

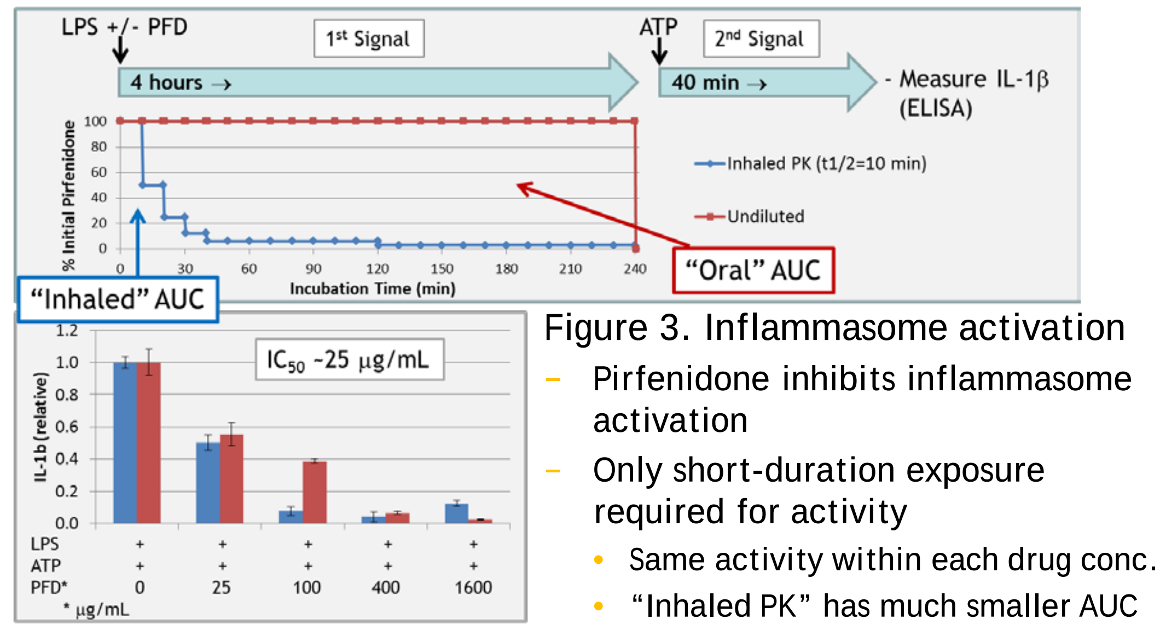


Figure 3. Inflammasome activation

- Pirfenidone inhibits inflammasome activation
- Only short-duration exposure required for activity
  - Same activity within each drug conc.
  - "Inhaled PK" has much smaller AUC

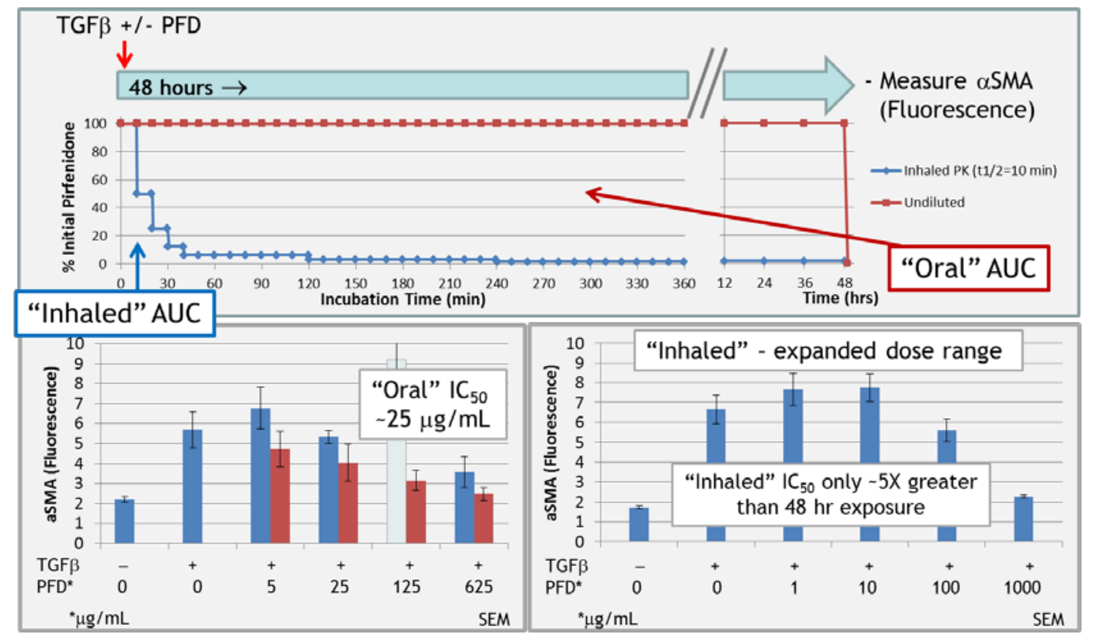


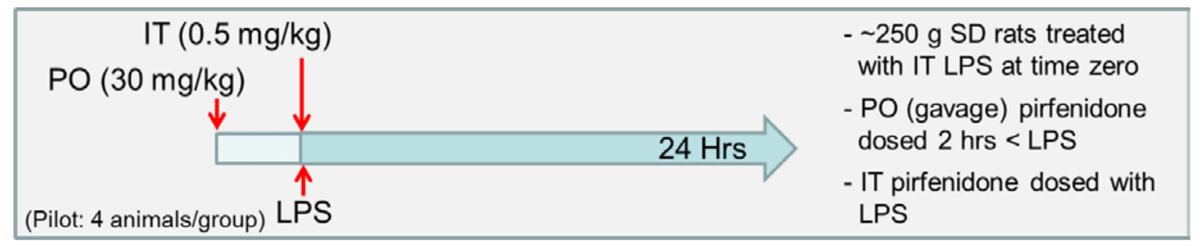
Figure 4. Fibroblast-to-myofibroblast differentiation

- Inhaled PK IC_{50} only ~5-fold greater than 48 hr exposure

RESULTS, cont.

3. Are small inhaled doses effective in animals?

LPS-induced inflammasome activation



- ~250 g SD rats treated with IT LPS at time zero
- PO (gavage) pirfenidone dosed 2 hrs < LPS
- IT pirfenidone dosed with LPS

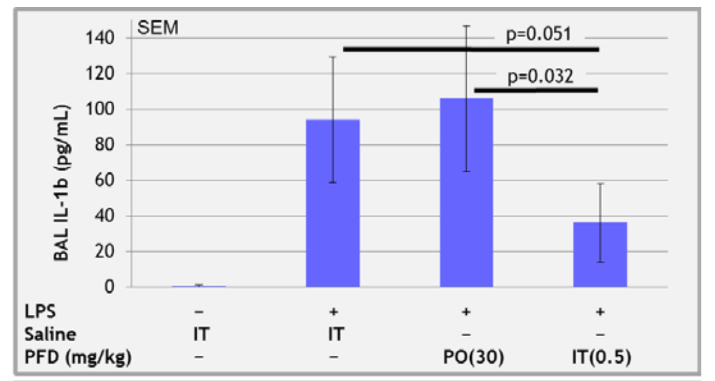


Figure 5a. LPS-induced IL-1β

- IT pirfenidone reduces IL-1β production
- IT significant over both control and 60-fold larger PO dose

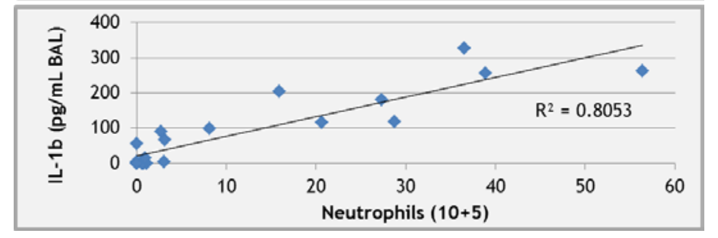
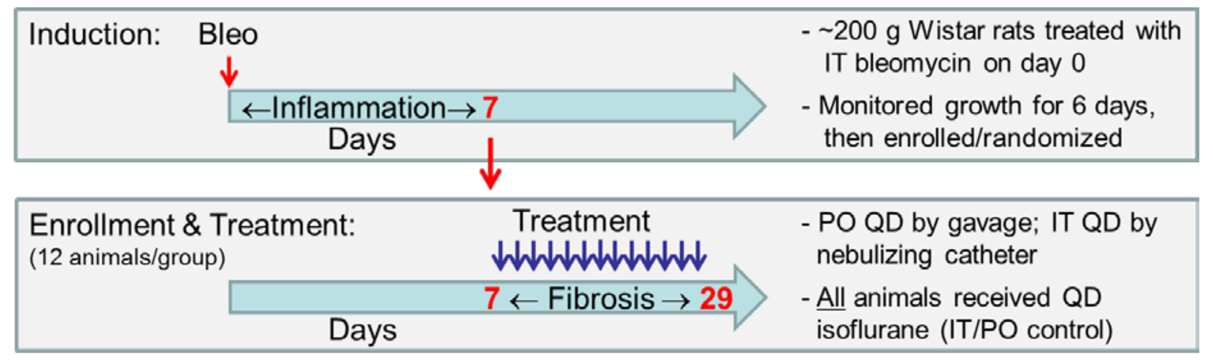


Figure 5b. LPS-induced inflammation

- IL-1β levels correlate to neutrophil infiltration

Bleomycin-induced pulmonary fibrosis



- ~200 g Wistar rats treated with IT bleomycin on day 0
- Monitored growth for 6 days, then enrolled/randomized

- PO QD by gavage; IT QD by nebulizing catheter
- All animals received QD isoflurane (IT/PO control)

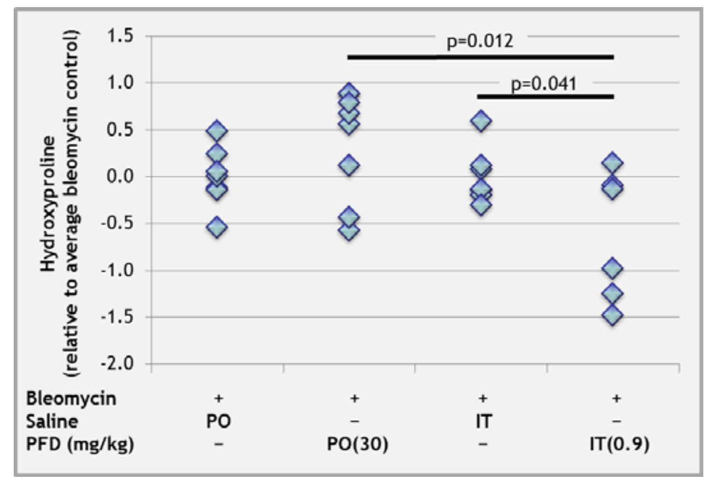


Figure 6. Bleomycin-induced hydroxyproline content (mg/right lung)

- IT pirfenidone reduces bleomycin-induced hydroxyproline content
- IT significant over bleomycin control and 33-fold larger PO dose

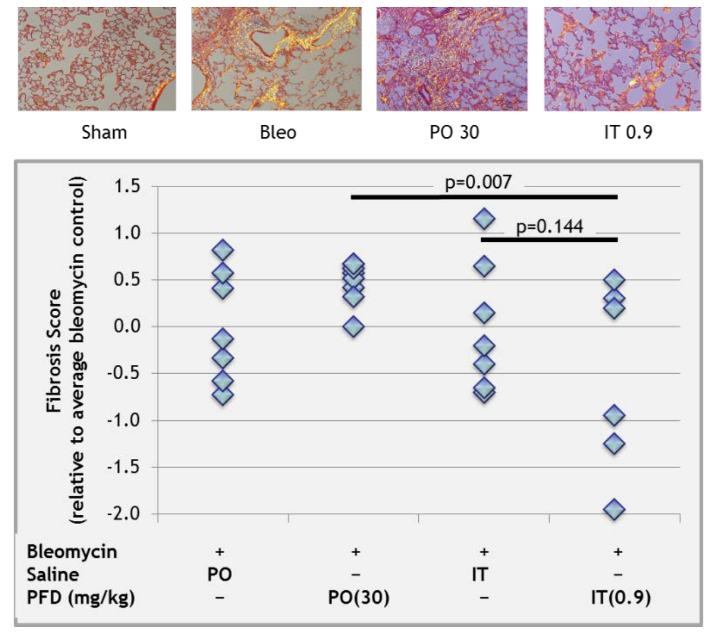
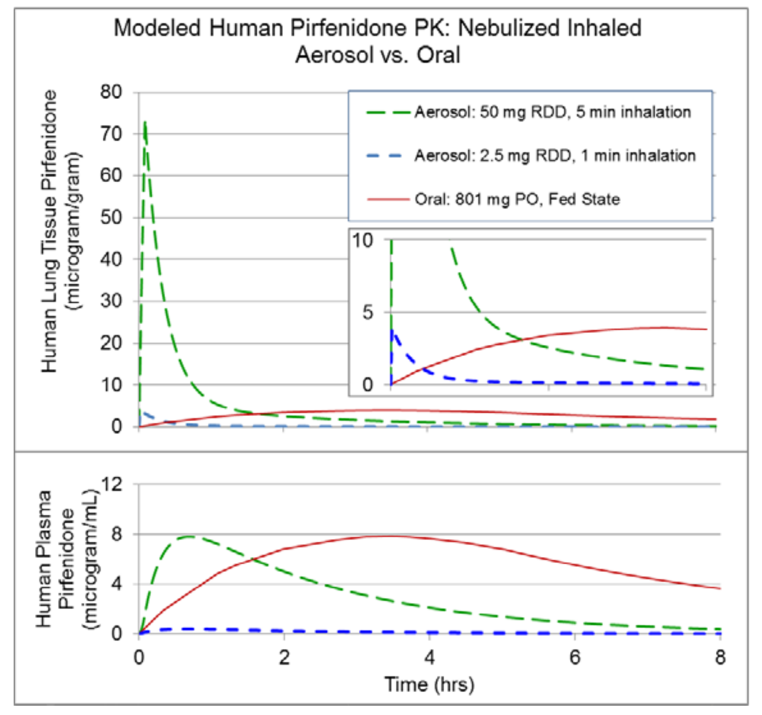


Figure 7. Bleomycin-induced pulmonary fibrosis

- IT pirfenidone reduces bleomycin-induced fibrosis score
- IT significant over 33-fold larger PO dose. Positive trend, but insignificant vs. bleomycin control

HUMAN MODELING



ELF: RDD = 25%, $t_{1/2}$ = 0.5 min Lung tissue: RDD = 50%, $t_{1/2}$ = 8.3 min Plasma: $t_{1/2}$ = 2.5 hrs	Inhaled (mg RDD)		Oral(6) (mg)
	2.5	50	801
Epithelial Lining Fluid (ELF)			
Cmax	12.0	526.8	~3.9
$AUC_{(0-18hr)}$	0.8	40.1	~30.8
Lung Tissue			
Cmax	3.9	73.1	3.9
$AUC_{(0-18hr)}$	1.7	34.6	30.8
Plasma			
Cmax	0.4	7.8	7.8
$AUC_{(0-18hr)}$	1.1	23.9	55.1

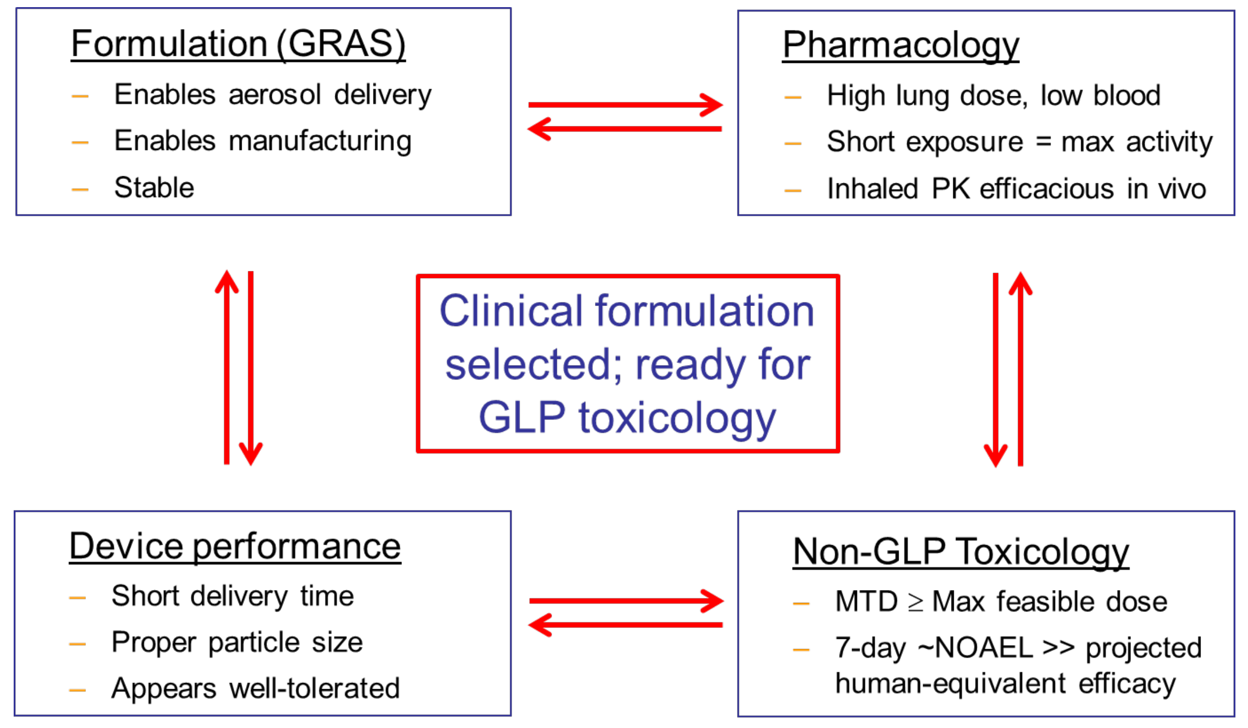
Figure 8. Human modeling: Tidal-inhaled vs. oral administration

- 2.5 mg RDD delivers same lung tissue Cmax as 801 mg oral dose with 20-fold and 50-fold lower plasma Cmax and AUC, respectively
- 50 mg RDD delivers same plasma Cmax as 801 mg oral dose with 19-fold larger lung tissue Cmax and 135-fold larger ELF Cmax

SUMMARY

1. Inhalation delivers a large lung Cmax with low blood levels
   - Relatively short-duration pirfenidone exposure (est. initial human pulmonary half-life ~8.3 minutes)
   - Model shows ~160-fold less device-loaded pirfenidone (5 mg for ~2.5 mg RDD) delivers equivalent lung Cmax as 801 mg oral dose
   - ~46-fold escalation possible before exceeding oral blood levels
2. Short-duration exposure sufficient for maximum activity
   - Enables use of inhaled pirfenidone Cmax advantage for IPF therapy
3. Are small inhaled doses effective in animals?
   - Inhaled efficacy stronger than larger oral dose levels
   - Supports favorable transition of inhaled therapy to IPF patients

CLINICAL CANDIDATE SELECTED



Formulation (GRAS)
- Enables aerosol delivery
- Enables manufacturing
- Stable

Pharmacology
- High lung dose, low blood
- Short exposure = max activity
- Inhaled PK efficacious in vivo

Clinical formulation selected; ready for GLP toxicology

Device performance
- Short delivery time
- Proper particle size
- Appears well-tolerated

Non-GLP Toxicology
- MTD ≥ Max feasible dose
- 7-day ~NOAEL >> projected human-equivalent efficacy

METHODS

Pharmacokinetics and Lung-Tissue Distribution:

To evaluate pirfenidone lung and plasma pharmacokinetics (PK), oral-dosed rats received pirfenidone by gavage at 45 mg/kg in 0.5% CMC (PO), and aerosol-dosed rats received intratracheal (IT) pirfenidone at 5 mg/kg. All IT administrations were performed using a Penn-Century Microspray® aerosolizer catheter. Plasma and lung tissue samples were assessed for pirfenidone content at various time points by LC/MS.

LPS-induced inflammasome activation:

264.7 macrophages were plated in a 96-well plate with a seeding density of 100,000 cells per well and incubated overnight. Following incubation, media was removed and replaced with media containing 0% FBS and either 200 ng/ml LPS alone or LPS with 0-1600 μg/mL pirfenidone. Cultures were incubated for 4 hrs, washed and replaced with media containing 2 mM ATP. After an additional 40 min incubation, supernatants were removed and evaluated for IL-1β content by ELISA. To evaluate the effect of pirfenidone exposure duration on inflammasome activation, cultures were exposed to pirfenidone for either the entire 4 hr LPS-incubation or quickly removed to mimic a 10 min inhaled half-life.

TGF-β1-induced fibroblast-to-myofibroblast differentiation:

Normal, primary human pulmonary fibroblasts were plated in a black 96-well collagen coated plate with a seeding density of 20,000 cells per well and incubated overnight. Following incubation, media was removed and replaced with media containing 0% FBS and either TGFβ1 alone or TGFβ1 with 0-1000 μg/mL pirfenidone. Cultures were then incubated for 48 hrs, and evaluated for alpha-smooth muscle actin (αSMA) production by fluorescent labeled antibody. To evaluate the effect of pirfenidone exposure duration on TGFβ1-mediated fibroblast-to-myofibroblast differentiation, cultures were exposed to pirfenidone for either the entire 48 hr incubation or quickly removed to mimic a 10 min inhaled half-life.

LPS-induced pulmonary inflammasome activation:

Sprague Dawley rats (200-250 grams) were administered a single dose of IT LPS using a intubation delivery device. Sham animals were treated with saline. A single pirfenidone dose was either delivered IT (0.5 mg/kg) in the 300 μL LPS dosing solution, or by gavage (PO; 30 mg/kg in 300 μL) 2 hours before LPS exposure. After 24 hours, animals were euthanized. Lungs were lavaged and bronchial lavage fluid (BAL) was assessed for total and differential cell count, and IL-1β content by ELISA.

Bleomycin-induced pulmonary fibrosis:

Wistar rats (175-225 grams) were administered IT bleomycin using a Penn Century MicroSprayer® catheter. On the seventh day following bleomycin exposure, animals initiated treatment with either saline or pirfenidone. Animals were dosed once a day on days 7 through 28. Pirfenidone was administered either IT using Penn Century MicroSprayer catheter (0.9 mg/kg) or by PO gavage (30 mg/kg). Sham and bleomycin control groups received either PO or IT saline by Penn Century MicroSprayer catheter. All study animals received once-a-day isoflurane. On day 29, animals were euthanized. Right lungs were extracted and measured for hydroxyproline content (mg/right lung). Left lungs were embedded, sectioned and stained with picrosirius red. Stained tissues were scored for lung tissue fibrosis. Twenty random photographs of each stained lung tissue section were taken, blinded and scored by an independent review panel. Observations were pooled for analysis.

Human pharmacokinetic modeling:

To estimate human pharmacokinetic parameters following a tidal-inhaled aerosol dose, a model was created from the following data and assumptions: 1. Human pulmonary elimination half-life is 2.5X the measured rat half-life (~8.3 min); 2. Inhaled aerosol particles less than 5 um (RDD) deposit in the lung; 3. Nebulizer aerosol performance characteristics (data not shown); 4. Human oral plasma pharmacokinetics(6); and 5. Inhaled aerosol dose is 100% bioavailable.

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