LRRK2 Kinase Inhibitors of Different Structural Classes Induce Abnormal Accumulation of Lamellar Bodies in Type II Pneumocytes in Non-Human Primates but are Reversible and Without Pulmonary Functional Consequences

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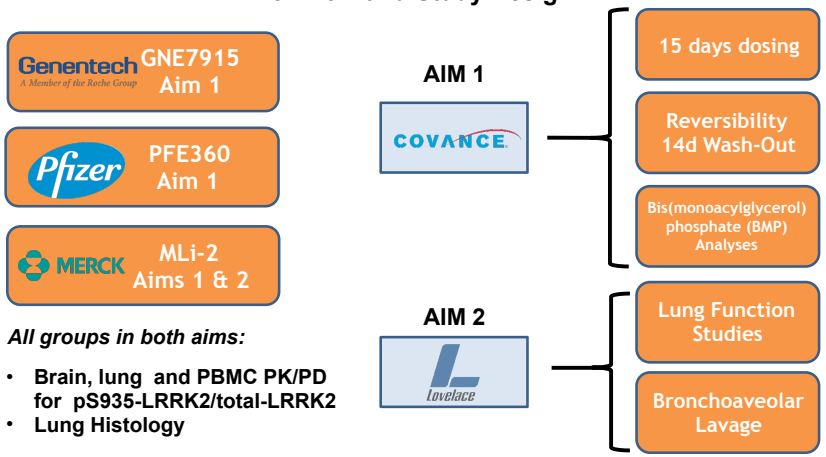
Rationale

- Mutations in the Leucine-rich repeat kinase 2 (LRRK2) that enhance kinase activity cause Parkinson's disease (PD). Kinase-enhancing mutations are neurotoxic, hence there is a strong therapeutic focus on discovery of LRRK2 kinase inhibitors capable of slowing the progression of PD.
- However, concerns of mechanism-based toxicity of LRRK2 inhibition arose following a report from Fuji et al.(2015) that showed LRRK2 kinase inhibitors caused morphologic changes in lungs of non-human primates (NHP) including abnormal accumulations of lamellar bodies in type II pneumocytes. Similar findings were reported earlier in LRRK2 knockout rodents.

There were two major aims for the present work :

1. **Explore the chemotype dependence and reversibility of NHP lung findings by comparing effects of structurally diverse LRRK2 kinase inhibitors. Correlate findings with exploratory biomarker.**
2. **Assess the impact of lung morphological changes on pulmonary function in NHPs**

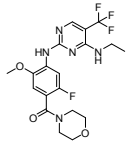
Workflow and Study Design



All groups in both aims:

- **Brain, lung and PBMC PK/PD for pS935-LRRK2/total-LRRK2**
- **Lung Histology**

LRRK2 Kinase Inhibitors: Structure and Properties

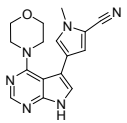


GNE7915

Unbound pS935 in vivo IC_{50} brain=97 nM

Off target kinase % inhibition >50% at 1 μM IVG: CHK2, CLK2, CamKIIalpha/delta, FAK, Fer, Fes, FLT3/4, GSK3beta, LTK, MLK1, PKD2/3, PhKgamma1, Rsk2/3, STK33, TAK1-TAB1, TSSK1, TTK

Off target receptor % inhibition >50% at 10 μM CEREP: 5-HT_{2B} IC50= 1000nM

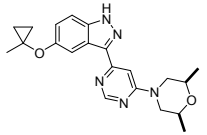


PFE360

Unbound pS935 in vivo brain IC_{50} =3 nM

Off target kinase % inhibition >50% at 1 μM ActivX: IRAK3, LOK, MST1/2, RSK2

Off target receptor % inhibition >50% at 10 μM Pfizer: 0 'hits'



MLi-2

Unbound pS935 in vivo IC_{50} brain=0.8 nM

Off target kinase % inhibition >50% at 1 μM IVG: CLK4, MAP3K14, MAP3K5, CLK2, and TTK

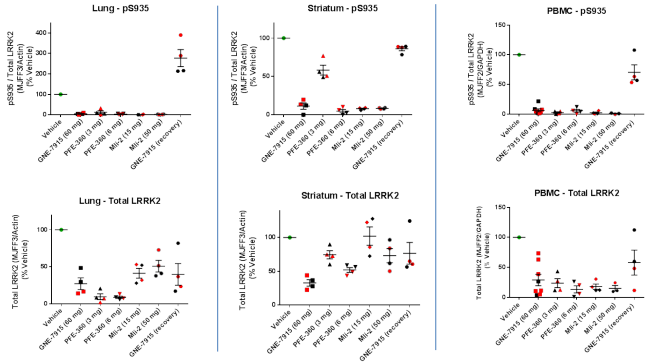
Off target receptor % inhibition >50% at 10 μM Panlabs: 5HT_{2B} IC50=1200nM

Aim1: Lung Histopathology, Reversibility and Dose Effect

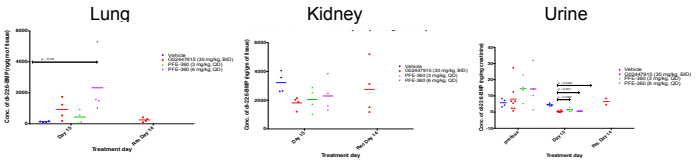
LRRK2 inhibitor	Animals per Group		Dose (mg/kg)	Targeted Exposure Multiples*	Observed Exposure Multiples#		Lung Type II Cell Vacuolation	
	M	F			M	F	M	F
GNE7915	2	2	30 BID	1x	3.2x	2.0x	2	2
	2	2	+14 day dose-free				0	0
PFE360	2	2	3 QD	2x	1x	1.2x	0	0
	2	2	6 QD	8x	10.2x	8.1x	2	2
MLi-2	2	2	15 QD	1x	9.5x	16.9x	0	0
	2	2	50 QD	10x	45.5x&	64.8x	1	2

*Targeted exposure multiples were based on predicted monkey unbound plasma AUC over 24 hours divided by mouse brain unbound IC50 concentrations for pS935 multiplied by 24. Mouse brain unbound IC_{50} concentrations for pS935 IC_{50}=97 nM for GNE7915, 0.8 nM of MLi-2, and 3 nM for PFE-360. #Observed exposure multiples are calculated as described above with actual PK data for monkey plasma AUC over 24 hours on Day 15.& One animal had low exposure (only 13.9x).

LRRK2 Kinase Inhibitors Reduce pS935 and Total LRRK2 Protein

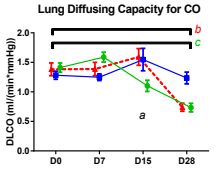


LRRK2 Kinase Inhibitors Increase di-22:6-BMP in Lung and Decrease Levels in Kidney and Urine

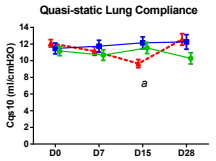


No changes seen in plasma and an increase was seen in brain at both doses of PFE360

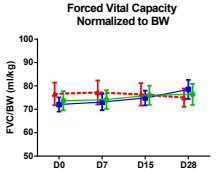
Aim 2: MLi-2 LRRK2 Kinase Inhibitor Has No Effect on Pulmonary Function



A two way ANOVA revealed a significant effect of time ($F_{(3, 120)}$ = 12.02, P < 0.001) but not treatment ($F_{(2,120)}$ = 1.14; NS) on lung diffusion capacity for CO. The vehicle group differed from MLi-2 50 mg/kg at day 15 (a: p<0.05, Bonferroni post test), however, the magnitude of change was not considered functionally relevant. Vehicle group differed from baseline at day 28 (b: p<0.01, Bonferroni post test) and MLi-2 50 mg/kg group also differed from baseline at day 28 (c: p<0.001, Bonferroni post test).



A two way ANOVA revealed no significant effect of time ($F_{(3, 120)}$ =0.58; NS) or treatment ($F_{(2,120)}$ =2.33; NS) on quasi-static lung compliance. Vehicle group differed from MLi-2 15 mg/kg only on day 15 (a: p<0.05, Bonferroni post test). However, the vehicle on D15 was significantly different for its own baseline (D0). Neither MLi-2 15 mg/kg nor MLi-2 50 mg/kg differed from their baseline at any time point.



A two way ANOVA revealed no significant effect of time ($F_{(3, 120)}$ =0.18; NS) or treatment ($F_{(2,120)}$ =0.16; NS) on forced vital capacity normalized to body weight.

Conclusions

- Three distinct LRRK2 kinase inhibitors produced the previously reported lung histopathology (mild accumulation of lamellar bodies in type II pneumocytes) in NHPs - confirming an on-target lung effect.
  - No morphologic effects were seen with any LRRK2 inhibitor in brain or kidney.
  - Di-22:6-BMP was lower in urine and kidney and increased in lung in all groups given LRRK2 inhibitors.
  - GNE7915 effects on lung and BMP were reversed after 14d washout.
- PFE360 and MLi-2 induced lung histologic effects only at high doses, despite both low and high dose groups at Cmax reducing pS935 in lung by >90%. Notably, the lower dose of MLi-2 still reduced LRRK2 pS935 at ~Cmax by >90%.
- MLi-2 effects on lung histology were not associated with functionally significant alterations in any pulmonary functional endpoint examined.
- Overall, these data suggest that the on target morphological changes observed in the lungs of LRRK2 kinase inhibitor treated NHPs may not prevent the clinical evaluation of the therapeutic potential of LRRK2 kinase inhibitors in PD.