LRRK2 DETECTION IN PBMC CONSORTIUM

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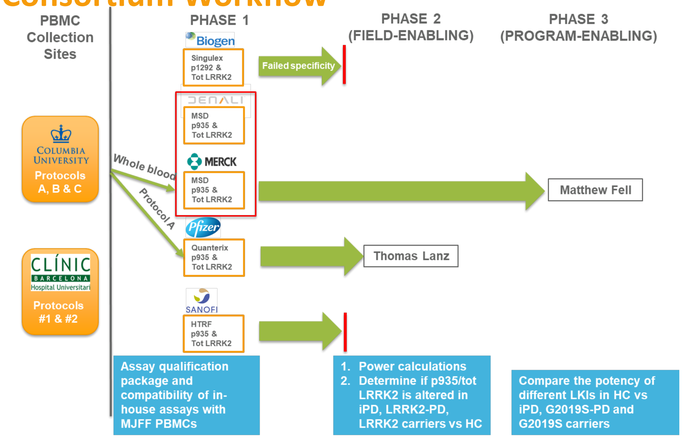
Introduction

The "LRRK2 Detection in PBMC Consortium" is a pre-competitive collaboration between MJFF and select industry partners with the goal of optimizing the measurement of pLRRK2 in human PBMCs. MJFF launched the consortium in response to the discussions at the LRRK2 Industry Summit in 2016. Each company provided in-kind analysis for pLRRK2 and total LRRK2 using in-house immunoassay platforms while PBMCs collected through the two MJFF PBMC biobanking initiatives served as the matrix for the analysis. The consortium activities were split into three phases and goals for each phase of the study were defined and refined through discussions with consortium members.

Consortium Members

MJFF	Shalini Padmanabhan, Samantha Hutten, Marco Baptista, Amasi Kumeh, Evelia Johnston, Alyssa Reimer & Ashwin Mallya
MJFF Advisors	Kalpana Merchant, Roy Alcalay & Dario Alessi
Clinical sites	Columbia University: Najah Levers, Chris Liong & Roy Alcalay Barcelona: María José Martí Domenech & Eduardo Tolosa
Biogen	Omar Mabrouk, Warren Hirst, Danielle Graham
Denali	Sarah Huntwork-Rodriguez, Stacy Henry & Carole Ho
Merck	Julie Lee, Payal Sheth, Matthew Fell & Matthew Kennedy
Pfizer	Thomas Lanz, David Gray, Donal Gorman, Alison Joyce Julie Coughlan, Michele Wolfe (Quanterix)
Sanofi	Nathalie Schussler, Sylviane Boularand, Eric Boitier, Jean-François Dedieu & Laurent Dubois

Consortium Workflow



Phase 2 sought to determine whether pS935 levels differ in iPD, HC, and G2019S manifesting and non-manifesting subjects.

Methods:

Sensitive and specific S935 and total LRRK2 assays were validated on the Quanterix Simoa platform in human PBMC lysate. Preliminary power calculations suggested that n=20 or more would enable quantitation of at least 30% difference between subjects at 80% power. Patient PBMCs lysates were prepared at Columbia and shipped (frozen) to Quanterix for blinded analysis (Columbia A protocol). Statistical analysis was performed at Pfizer.

Table 1. Quanterix assay characteristics

Assay	Capture Ab	Detection Ab	Standard	LLOD	Variability (%CV)
Human S935 LRRK2	Neuromab N241A/34	Abcam clone UDD2	Life Technologies WT LRRK2 cat# A15197	4.2 pg/mL*	Both intra and inter-assay %CV's <15%
Human Total LRRK2	Neuromab N241A/34	Cell Signaling Technologies D18E12	Life Technologies WT LRRK2 cat# A15197	19 pg/mL*	Intra-assay %CV < 15%; inter-assay %CV < 20%

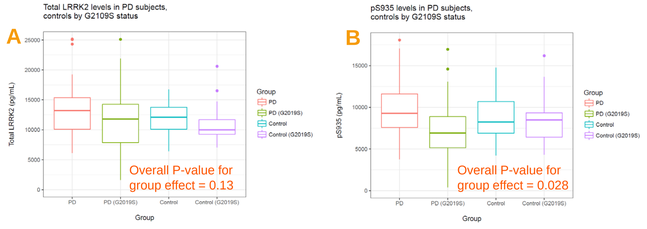
Lysis buffer: 50 mM Tris-HCL, 150 mM NaCL, 1% Triton x-100, 2% Glycerol, 10 mM PPA, 20 mM NaF, 2 mM Na3VO4, 2 mM EGTA, 2 mM EDTA, HALT protease and phosphatase inhibitor cocktail, pH 7.5

Table 2. Summary subject information

Clinical Characteristic		Control (G2019S-) (n = 22)	G2019S+ non-PD (n = 16)	PD (G2019S-) (n = 46)	PD (G2019S+) (n = 33)
Age (Years):	Mean (SD)	69.0 (7.1)	57.9 (11.6)	64.9 (9.4)	71.7 (8.9)
	Range	57-85	37-83	41-82	56-91
Gender:	Male	13 (59%)	6 (38%)	29 (63%)	19 (58%)
	Female	9 (41%)	10 (62%)	17 (37%)	14 (42%)
Disease Duration (Years):	Median (IQR)			7 (2.25-10)	11 (7-15)
	Range			0-25	0-26
Total UPDRS*	Mean (SD)			28.1 (16.4)	34.4 (18.7)
	Range			8-94	4-81
UPDRS (Part 3)*	Mean (SD)			19.6 (12.3)	22.2 (11.8)
	Range			5-64	1-53

*Note: UPDRS data were only available for 80% of PD subjects.

Figure 1. Total (A) and S935 (B) LRRK2 levels measured in patient PBMCs.



Y-axis = LRRK2 levels interpolated off of a standard curve of recombinant LRRK2. Red = PD (G2019S-), green = PD (G2019S+), teal = control (G2019S-), purple = control (G2019S+). Significant differences between groups were only detected in S935 LRRK2.

Table 3. Summary of differences between groups in total or S935 LRRK2 levels.

Comparison	P-value (Total LRRK2)	% change (Total LRRK2)	P-value (LRRK2 G2019S)	% change (LRRK2 G2019S)
PD vs. Control	0.13	+15%	0.28	+11%
PD (G2019S) vs. Control (G2019S)	0.75	+4%	0.15	-17%
PD vs. PD (G2019S)	0.06	+16%	**0.003**	**+32%**
Control vs. Control (G2019S)	0.67	+5%	0.95	-1%

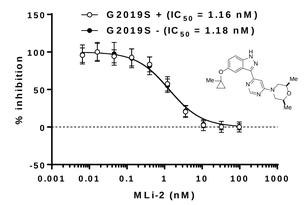
The only significant differential effect was the comparison of G2019S- to G2019S+ PD subjects, with the latter having reduced S935 LRRK2. Age, gender, disease duration and UPDRS had no significant impact on total or S935 LRRK2 as covariates.

Phase 3 sought to determine the potency of three LRRK2 kinase inhibitor tool compounds across the G2019S carrier and non-carrier groups using Merck's MSD pS935 assay.

Methods:

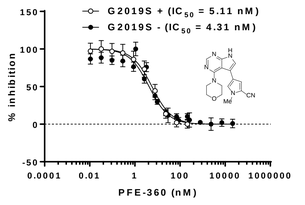
pSer935 & total LRRK2 assays developed on Meso Scale Discovery (MSD) platform. Whole blood was collected from healthy controls (n=6), idiopathic PD patients (n=7), PD patients with G2019S mutations (n=8) and unaffected G2019S mutation carriers (n=4). Whole blood was couriered from Columbia University to Merck (Kenilworth NJ) for same day PBMC isolation and incubation (90 minutes) with LRRK2 kinase inhibitors (MLi-2, PFE-360, GNE-7915 and MLi-X). LRRK2 pSer935 inhibitory potency was determined in duplicate for each donor.

MLi-2



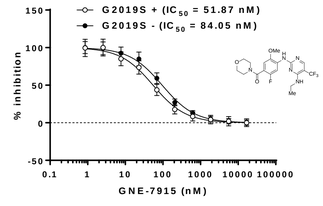
Diagnosis	Genotype	Subjects	IC_{50} (nM)	SD	SE
Control	G2019S -	n = 6	1.41	0.46	0.19
PD	G2019S -	n = 7	0.99	0.26	0.10
Control	G2019S +	n = 4	1.13	0.30	0.15
PD	G2019S +	n = 8	1.19	0.17	0.06

PFE-360



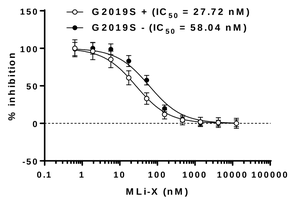
Diagnosis	Genotype	Subjects	IC_{50} (nM)	SD	SE
Control	G2019S -	n = 6	4.10	1.58	0.64
PD	G2019S -	n = 7	4.52	1.06	0.40
Control	G2019S +	n = 4	5.24	1.42	0.71
PD	G2019S +	n = 8	4.98	0.96	0.34

GNE-7915



Diagnosis	Genotype	Subjects	IC_{50} (nM)	SD	SE
Control	G2019S -	n = 6	91.90	26.57	10.85
PD	G2019S -	n = 7	76.88	19.55	7.39
Control	G2019S +	n = 4	55.39	19.19	9.60
PD	G2019S +	n = 8	48.58	6.78	2.40

MLi-X



Diagnosis	Genotype	Subjects	IC_{50} (nM)	SD	SE
Control	G2019S -	n = 6	68.82	20.01	8.17
PD	G2019S -	n = 7	50.15	7.9	2.98
Control	G2019S +	n = 4	25.42	3.81	1.90
PD	G2019S +	n = 8	28.94	14.24	5.04

Summary and next steps

- **Phase 2 summary: Total and S935 LRRK2 levels were similar between PD and control PBMCs overall. In subjects with PD (but not healthy controls), G2019S PBMCs had 32% lower S935 LRRK2 levels. Remaining samples have been sent to Denali for pRab10 measurement.**
- **Phase 3 summary: Demonstrated the potential to observe genotype dependent shifts in LRRK2 inhibitor potency (based on pSer935) in human PBMCs that are likely chemotype specific (G2019S to WT shift observed for MLi-X but not MLi-2, PFE-360 or GNE-7915). Ex-vivo LRRK2 inhibitor potency (based on pSer935) in Human PBMCs is consistent with data obtained from Merck's ex-vivo PBMC assay in WT and G2019S KI mice.**
- **The Consortium is currently discussing the potential to expand on these findings in other matrices using the recently developed Rab antibodies to optimize and develop new target engagement and patient enrichment biomarkers.**