The "LRRK2 Detection in PBMC Consortium" is a pre-competitive collaboration between MJFF and select 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 activity between MJFF and select industry partners with the goal of collaboration between MJFF and select industry partners with the goal of collaboration between MJFF and select industry partners with the goal of collaboration between MJFF and select industry partners with the goal of collaboration between MJFF and select industry partners with the goal of.

**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 ≥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.

**Phase 3** sought to determine the potency of three LRRK2 kinase inhibitor tools 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.

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- **Denali**
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- **Merck**
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- **Pfizer**
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**Consortium Workflow**
- **PBMC Collection Sites**
  - PHASE 1 (PRE-ENROLLING)
    - PHASE 2 (FIELD-ENROLLING)
    - PHASE 3 (PROGRAM-ENROLLING)

**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.