



PARKINSON'S BIOCHEMICAL BIOMARKERS PIPELINE REPORT

October 2025 (*Version 3*)

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Report Objectives

This report provides an overview of the Parkinson's Disease (PD) biomarkers landscape, with a focus on biochemical and molecular based assays that MJFF staff is monitoring. It is intended to reflect biomarkers at various stages of development and validation for the indicated intended uses (e.g., diagnosis, monitoring, etc.) Although MJFF and the field are actively pursuing discovery-focused approaches using 'omics and multiplexed platforms, these will not be included in this report until key 'hits' have been identified for which quantitative assays can be developed and moved through this pipeline. Finally, some biomarkers currently under development are not reflected here due to confidentiality.

Internally, MJFF is leveraging this report to identify critical gaps in our portfolio and monitor progression of promising measures moving towards qualification. This will help shape MJFF's biomarkers vision and strategy and ensure transparency and communication across efforts.

Externally, this report will serve as a resource to our partners who may be interested in MJFF's biomarkers portfolio and opinion on the state of the field. It could also be used to help determine which biomarker(s) may be relevant to include as exploratory endpoints in clinical trials.

The report will be updated and distributed on an annual basis.

If you have any comments or suggestions for this report, please contact MJFF at biomarkers@michaeljfox.org

TERMINOLOGY & DEFINITIONS BASED ON FDA'S BEST GUIDELINE

Stage: *What stage of development the measure is in? (the stage noted indicates completion of the previous stage(s))*

- **Development** – Early work to identify a putative biomarkers (ahead of clinical testing)
- **Optimization** - Refinement of a previously-identified molecule or analyte measurement (ex. chemistry optimization, changing antibody pairs, optimizing the assay to a different platform or biofluid, adapting the biomarker for PD (from another neurodegenerative field))
- **Analytical validation** – Rigorous characterization of the measure resulting in the establishment of standard operating procedures (ex. effect of pre-analytical variables, sensitivity, specificity, inter- and intra- assay variability in intended biological matrix)
- **Initial clinical qualification** – Testing the biomarker in an appropriate clinical sample set/clinical study to establish its utility for the intended use (diagnostic, prognostic, monitoring, pharmacodynamic, etc.). Assays evaluated at this stage already have strong validation parameters, biologic plausibility, and potential claims for one of the intended uses. This category does NOT indicate regulatory qualification.

Note: Multiple research groups may be working on development of the same biomarker, the status of the most robust is reflected herein.

Intended Use: *What is the intended and/or potential use of the measure for Parkinson's disease? (definitions from FDA BEST, see Appendix)*

- **Diagnostic** – detects or confirms presence of PD or identifies individuals with a subtype of the disease/stage of the disease
- **Prognostic** – identifies the likelihood of a clinical event, stage transition, disease recurrence, or progression in people with PD or prodromal PD
- **Predictive** – identifies patients who are more likely to respond to certain treatments
- **Susceptibility/risk** – indicates the potential for developing PD in those who do not currently have clinically apparent disease (ex. phenoconversion)
- **Monitoring** – changes longitudinally and correlates with biomarkers of clinical progression of the disease at any stage (ex. prodromal, *de novo*, etc.)
- **Pharmacodynamic** – shows a biological response to an intervention/exposure that may or may not be related to PD pathophysiology
- **Safety** – Indicates the presence or extent of toxicity related to an intervention/exposure

MJFF has referenced the above FDA BEST definitions to classify each measure, but assignments can be subjective

A note about annotations & footnotes:

- MJFF has provided footnotes to justify staging & high-level results, where deemed necessary
- Annotations for qualified pharmacodynamic intended uses are not included
- References related to qualification indicate that stud(ies) have been completed and results are as indicated
- Line items without annotations or footnotes are ongoing
- Please note that the 'qualified' annotation in this report is not meant to denote formal regulatory qualification for PD use





Overview of the Current Parkinson's Biochemical Biomarkers Pipeline

BIOMARKERS BY TARGET/PATHWAY

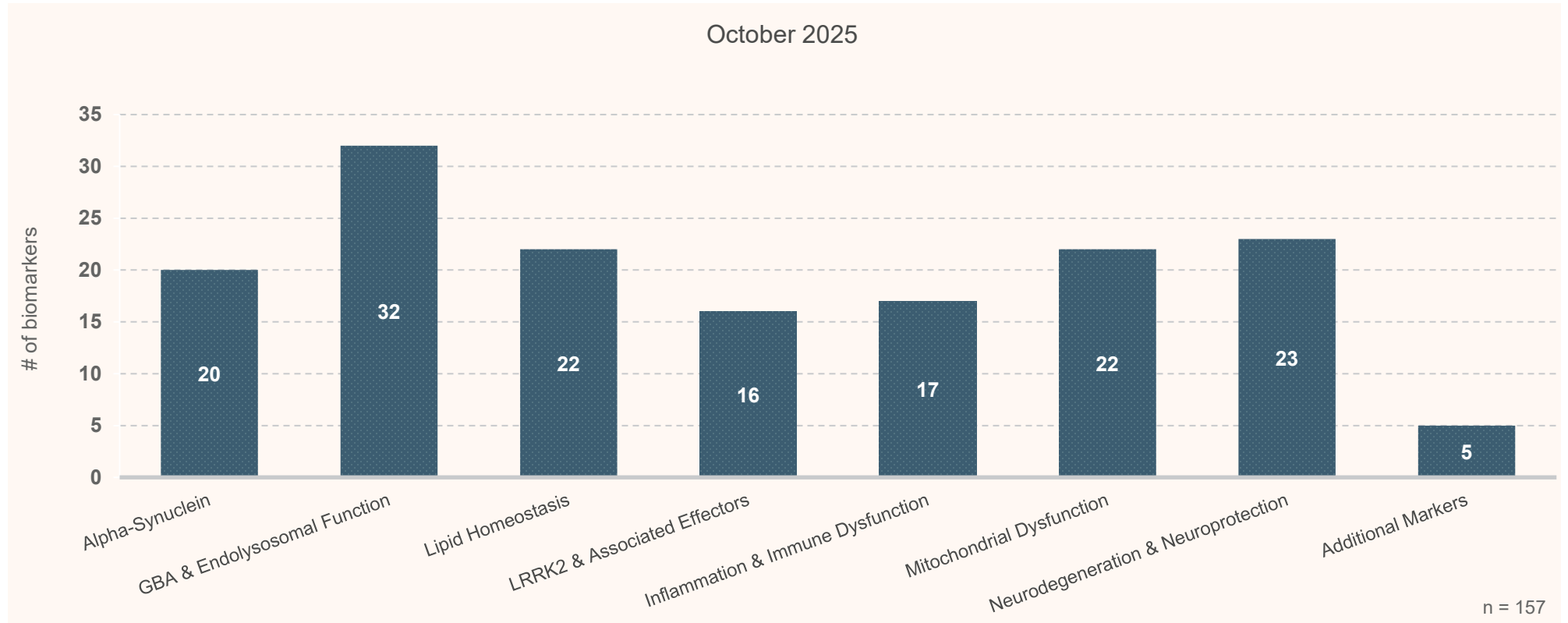


Figure 1: Number of target-based biomarkers by pathway that are currently ongoing across all stages (development, optimization, validation, qualification) or have passed all stages and were qualified for at least one use. Analytes in different biomatrices are counted as independent biomarkers (i.e., a measure in CSF and plasma is counted twice). Biomarkers that failed qualification are still counted only if there is ongoing development for another use or if qualification was inconclusive and additional work is needed.



BIOMARKERS BY MATRIX

October 2025

- CSF
- Plasma
- Specific Cell Types¹
- Serum
- Urine
- Other Blood Products²
- Brain-Derived Exosomes
- Tissues³
- Tears
- Saliva

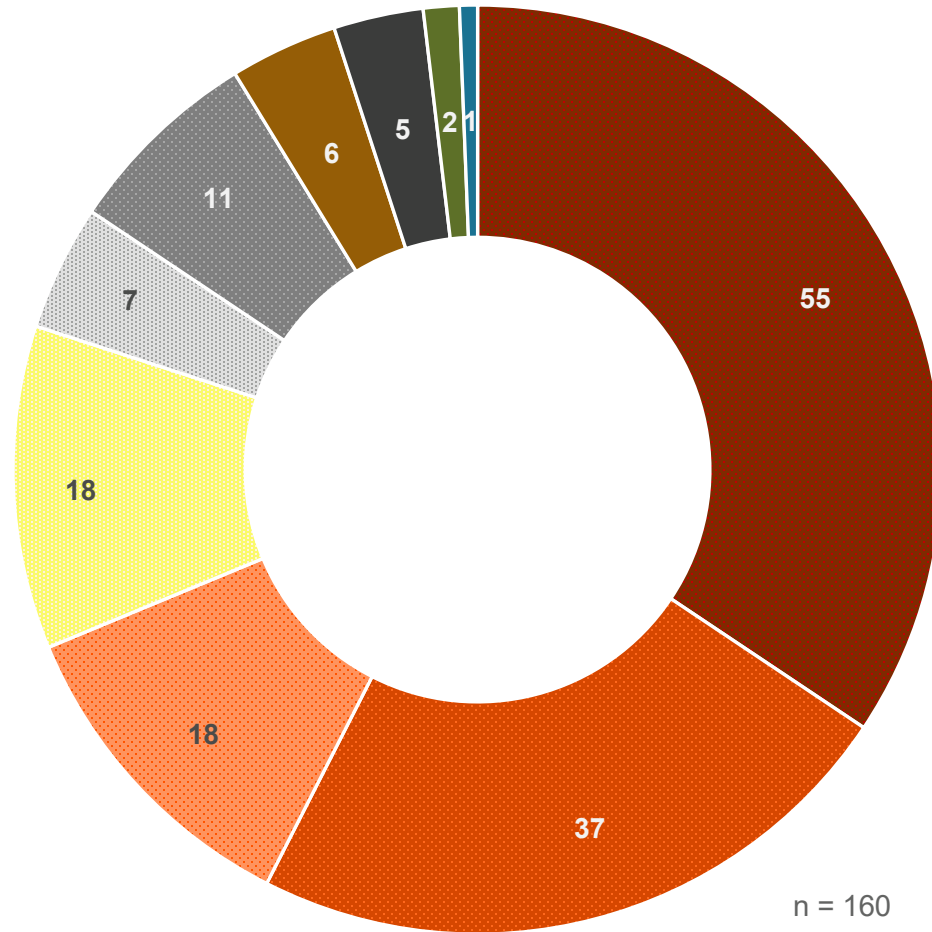


Figure 2: Number of biomarkers across different matrices. Markers that are being developed across multiple targets will be counted more than once (e.g., the same biomarker in CSF and plasma is listed twice). Inclusive of all biomarkers with ongoing efforts, those that have been qualified, or those with inconclusive/ additional work needed.

¹Includes PBMCs, B cells, monocytes and neutrophils

²Includes whole blood, buffy coat and dried blood spots

³Includes skin, muscle and olfactory mucosa



PROGRESS THROUGH THE PIPELINE

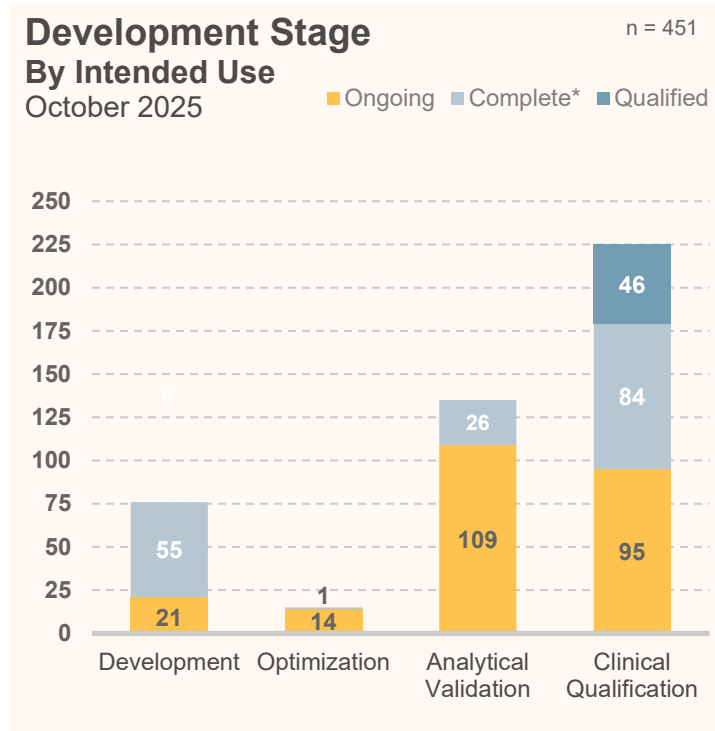


Figure 3: Biomarkers that are being developed across multiple matrices will be counted more than once (e.g., a biomarker in both CSF and plasma will be listed twice). **All** biomarkers are counted, including those that were not validated/qualified for intended use

*Complete includes not qualified, inconclusive, partially qualified or completed an earlier stage and did not move forward to another stage

Measures that have been initially qualified for specified intended uses in PD research:

Diagnostic

- Aggregated aSyn (CSF)
- Phospho-aSyn (pS129, skin)
- Neurofilament light (serum)*

Prognostic

- Glucosylceramide : Sphingomyelin ratio (CSF)
- Urate (CSF, plasma & serum)
- ApoA1 (plasma & serum)
- Aβ42 (CSF)
- Cortisol (plasma)
- IGF-1 (serum)
- Total tau (CSF)
- Phospho-tau (pT181, CSF)

Predictive

- GCCase activity (plasma & whole blood)
- GPNMB (CSF & plasma)
- BMP (urine)
- Lyso-Gb1 (dried blood)

Monitoring

- Glucosylceramide : Sphingomyelin ratio (CSF)
- Phospho-ubiquitin (pS65, CSF & plasma)
- Aβ42 (CSF)
- Neurofilament light (CSF & serum)

Susceptibility/Risk

- GCCase activity (plasma)
- Urate (plasma)

Pharmacodynamic

- Total aSyn (CSF & Plasma)
- GCCase protein (CSF & plasma) & GCCase activity (dried & whole blood)
- BMP (CSF & urine)
- GluCer (CSF & plasma)
- Total LRRK2 (CSF)
- Phospho-LRRK2 (pS935, PBMCs & whole blood)
- pRab10 (CSF & PBMCs)
- Urate (CSF, plasma & serum)

*differential diagnostic between synucleinopathies

Although some biomarkers have been **initially** qualified as fit-for-purpose, the PD field still suffers from a lack of progression & diagnostically relevant markers with clear, implementable cut-offs for use in patient care & clinical trials. Please note that the ‘qualified’ annotation in this report is not meant to denote formal regulatory qualification for PD use.



Biomarkers by Target/Pathway

ALPHA-SYNUCLEIN

Overview

Alpha-synuclein (aSyn) plays a critical role in Parkinson's disease (PD) pathogenesis and is a promising biomarker in PD and other synucleinopathies. aSyn is readily available in the extracellular space and is detectable in cerebral spinal fluid (CSF), blood, tears and saliva, in addition to peripheral tissues (skin, submandibular gland, olfactory mucosa and GI tract). In addition to total aSyn levels, increasingly more effort is dedicated to measuring aggregated and post-translationally modified forms of aSyn, aSyn content in extracellular vesicles (EVs), and aSyn associated with lipids, for use both as disease and pharmacodynamic biomarkers. A significant focus of MJFF's [Quantitative Biomarkers Program](#) (QBio) is dedicated to advancing objective measures of aSyn to support clinical trials.

MJFF Perspective & Unmet Need

Commercially available 'total' aSyn assays have been evaluated in large PD cohorts and are commonly used in clinical studies, but the breadth of post-translational modifications (PTMs) of aSyn has made it challenging to develop assays that truly capture the full diversity of aSyn species in human biospecimens. In addition, there remains a lack of knowledge of key pathogenic form(s) of aSyn. A quantitative map of aSyn PTMs in health and disease is lacking; therefore, only a few of the more prevalent aSyn PTMs (ex. phosphorylation and nitration) have been the focus of assay development to date. Several challenges have hampered these efforts, including lack of sensitive and specific antibodies and the effect of pre-analytical variables on these PTMs. Aggregated aSyn remains a top priority biomarker as it is closely linked to disease pathology and progression, at least in brain. Immunodetection of aggregated aSyn in biofluids has been difficult given its low concentration and the fact that "conformational-specific antibodies" tend to also bind to non-aggregated forms of aSyn (albeit with lower affinity). Measurement of aggregated aSyn in biofluids is usually below the limit of detection for even the most sensitive platforms. Aggregated aSyn in peripheral tissues is more promising and has been successfully measured using immunohistochemical approaches. A new skin biopsy test has recently reported high sensitivity and specificity to identify individuals with PD and evaluation for other intended uses is ongoing. Seed amplification assays (SAA), based on the ability of aggregated aSyn to also induce aggregation of monomeric aSyn, have been developed and validated. CSF SAA has been shown to be highly sensitive and specific as a diagnostic biomarker, with an FDA Letter of Support for use in clinical trial enrichment. Critical limitations for SAAs include lack of quantitation (current assays are binary), and optimization for less-invasive biomatrices (skin is most promising so far).



ALPHA-SYNUCLEIN

| Biomarker | Matrix | Assay Modality | Intended Use | Stage | | | | MJFF Role | |
|--------------|-----------------------------------------|----------------|---------------------------|-------------|--------------|-----------------------|------------------------------|-----------|--|
| | | | | Development | Optimization | Analytical Validation | Clinical Qualification | | |
| "Total" aSyn | CSF | Immunoassay | Diagnostic | | | | Did Not Qualify ¹ | | |
| | | | Prognostic | | | | Did Not Qualify ¹ | | |
| | | | Predictive | | | | Did Not Qualify ¹ | | |
| | | | Monitoring | | | | Did Not Qualify ¹ | | |
| | | | Pharmacodynamic | | | | Qualified | | |
| | Plasma | | Diagnostic | | | | Did Not Qualify ² | | |
| | | | Prognostic | | | | Did Not Qualify ² | | |
| | | | Predictive | | | | Did Not Qualify ² | | |
| | | | Monitoring | | | | Did Not Qualify ² | | |
| | Plasma (brain-derived EVs) ³ | | Diagnostic | | | | | | |
| | | | Prognostic | | | | | | |
| | | | Serum (brain-derived EVs) | Diagnostic | | | | | |
| | | | | Prognostic | | | | | |

¹Significant reduction in CSF total aSyn in PD, but too much variability across subjects to qualify for any intended use. In the absence of newer assays, no additional work recommended, focus on identifying other pathologically relevant species (e.g., pS129). Total aSyn may be a relevant normalization benchmark for modified aSyn assays.

²Total-aSyn in non-CSF biofluids does not correlate with central levels. No immediate next steps recommended unless a species of aSyn that is not predominant in red blood cells can be identified & assay developed. Focus on identifying other pathologically relevant species (e.g., pS129) in exosome-derived fractions.

³Assays to measure membrane-associated aSyn from plasma extracellular vesicles (EVs) are also being evaluated.



= Development unsuccessful



= Samples Provided



= MJFF Funding



= Consortia

ALPHA-SYNUCLEIN CONT.

| Biomarker | Matrix | Assay Modality | Intended Use | Stage | | | | MJFF Role |
|-------------------------|--------|----------------|-----------------|-----------------------|--------------|-----------------------|------------------------|-----------|
| | | | | Development | Optimization | Analytical Validation | Clinical Qualification | |
| "Total" aSyn (Cont.) | Saliva | Immunoassay | Diagnostic | Complete ¹ | | | | |
| | | | Prognostic | Complete ¹ | | | | |
| | Tears | | Diagnostic | | | | | - |
| | | | Prognostic | | | | | |
| | | | Predictive | | | | | |
| | | | Monitoring | | | | | |
| | | | Pharmacodynamic | | | | | |
| aSyn nanobodies | Plasma | Immunoassay | Diagnostic | | | | | |

¹Total-aSyn in non-CSF biofluids does not correlate with central levels. No immediate next steps recommended unless a species of aSyn that is not predominant in red blood cells can be identified & assay developed. Focus on identifying other pathologically relevant species (e.g., pS129) in exosome-derived fractions.

ALPHA-SYNUCLEIN CONT.

| Biomarker | Matrix | Assay Modality | Intended Use | Stage | | | | MJFF Role |
|-----------------|----------------------------|----------------------------------------------|---------------------|------------------------|--------------|-----------------------|------------------------|-----------|
| | | | | Development | Optimization | Analytical Validation | Clinical Qualification | |
| Aggregated aSyn | CSF | aSyn Seed Amplification Assay (SAA) | Diagnostic | Qualified ¹ | | | | |
| | | | Prognostic | | | | | |
| | | | Predictive | | | | | |
| | | | Susceptibility/Risk | | | | | |
| | | | Monitoring | | | | | |
| | | | Pharmacodynamic | | | | | |
| | Plasma (brain-derived EVs) | | Diagnostic | | | | | |
| | Olfactory mucosa | | Diagnostic | | | | | |
| | Skin | | Diagnostic | | | | | |
| | CSF | Digital aSyn Seed Amplification Assay (dSAA) | Diagnostic | | | | | |
| | CSF | Immunoassay | Diagnostic | | | | | |
| | Tears ² | | Diagnostic | Complete ² | | | | |
| | | | Monitoring | Complete ² | | | | |
| | Blood | Proximity ligation assay (PLA) ³ | Diagnostic | | | | | |
| | Monitoring | | | | | | | |
| Skin | Diagnostic | | | | | | | |
| | | Monitoring | | | | | | |

¹Various studies demonstrate high sensitivity & specificity of aSyn SAA in CSF for discriminating PD from controls and FDA has issued a Letter of Support for use of the assay for clinical trial enrichment. Data support higher SAA positivity in prodromal subjects and a high correlation with hyposmia, and lower SAA positivity in LRRK2 carriers. Investigation into PD subgroups and longitudinal changes are still ongoing. Other matrices are still in earlier stages of assay development.

²Independent replication and additional assay validation required to evaluate utility of measuring aggregated aSyn in tears.

³PLA for aggregated aSyn sensitively reveals diffuse, non-Lewy body aSyn oligomers in PD often missed by standard immunohistochemistry. In LRRK2 brains—including Lewy-body-negative cases—PLA shows widespread oligomeric/non-inclusion aSyn pathology, suggesting oligomers might accumulate without progressing to classic Lewy pathology in certain populations.

ALPHA-SYNUCLEIN CONT.

| Biomarker | Matrix | Assay Modality | Intended Use | Stage | | | | MJFF Role |
|-----------------------------|---------------------|----------------------|-----------------|------------------------------|--------------|-----------------------|------------------------|-----------|
| | | | | Development | Optimization | Analytical Validation | Clinical Qualification | |
| Nitrated aSyn | CSF | Immunoassay | Diagnostic | | | | | \$ |
| | | | Pharmacodynamic | | | | | |
| | Serum | | Diagnostic | | | | | |
| | | | Pharmacodynamic | | | | | |
| Phosphorylated aSyn (pS129) | CSF | Immunoassay | Diagnostic | | | | | \$ |
| | Colon | Immunohistochemistry | Diagnostic | Did Not Qualify ¹ | | | | \$ |
| | | | Prognostic | Did Not Qualify ¹ | | | | |
| | Submandibular gland | | Diagnostic | Did Not Qualify ¹ | | | | \$ |
| | | | Prognostic | Did Not Qualify ¹ | | | | |
| | Skin | | Diagnostic | Qualified ¹ | | | | - |
| | | | Pharmacodynamic | | | | | \$ |
| | | | Predictive | | | | | |
| Monitoring | | | | | | | \$ | |

¹In MJFF's S4 Cohort, pS129 aSyn in skin, colon and submandibular gland was not a highly sensitive marker (difficulty acquiring adequate tissue samples and varying results depending on preanalytical variables and analytical methodology). More recently, the SynOne test using 3 skin biopsies has been reported as having high specificity and sensitivity to identify aSyn pathology in skin. Current efforts to expand pS129-aSyn for different contexts of use are ongoing.

GBA, ENDOLYSOSOMAL FUNCTION & LIPID HOMEOSTASIS

Overview

Mutations in the GBA1 gene represent the most common genetic risk factor for Parkinson's disease (PD), and various therapeutic targeting strategies for its encoded protein, glucocerebrosidase β (GCase), including increasing levels, enhancing enzymatic activity, and reducing associated lipid substrates, are in the clinic. GCase converts glucosylceramide to ceramide, key components of a family of bioactive sphingolipids that regulate biological functions affected in PD, including mitochondrial function, autophagy, lysosomal function, and endosomal trafficking. Assays originally developed for Gaucher's Disease have been leveraged to evaluate GBA pathway biomarkers, lipid species, and substrates for the purposes of target engagement and patient stratification in PD. Mass-spectrometry panels have emerged to enable simultaneous quantitation of multiple lipid species in various biofluids. Relatedly, lysosomal dysfunction has been implicated in PD more broadly, and thus biomarkers associated with this pathway may also be relevant for patient stratification and to elucidate disease progression.

MJFF Perspective & Unmet Need

There are several inherent challenges with measuring GCase in biofluids: 1) it is not typically secreted, thereby rendering it difficult to interpret the relevance of measurements in CSF/blood, 2) it loses activity at pH levels typically found in blood and CSF, and 3) it can be difficult to dissect GBA1 from other glucosylceramidase family enzymes. There are multiple analytically validated GCase1 activity assays, which differ in substrate, pH, and extraction methods, making it challenging to compare different studies, or to properly interpret the data. Moreover, the activity assays mostly confirm a genotype effect, and ongoing efforts focus on more targeted blood cell populations to identify idiopathic PD patients who would benefit from GCase therapies. In addition to assessing GCase activity, the field is moving towards evaluation of other upstream and downstream targets within the glycosphingolipid pathway to assess changes at an individual lipid level and overall parameters of lysosomal health and function. Finally, given the role of lysosomal and protein clearance dysfunction in PD, a robust toolbox of lysosomal biomarkers is needed, both as part of a precision medicine approach and also to support known and emerging lysosomal-targeted therapeutics.



GBA & ENDOLYSOSOMAL FUNCTION

| Biomarker | Matrix | Assay Modality | Intended Use | Stage | | | | MJFF Role |
|------------------------------------------------|----------------------------|-------------------|---------------------|-------------|--------------|-----------------------|------------------------|-----------|
| | | | | Development | Optimization | Analytical Validation | Clinical Qualification | |
| Lysosomal cation-transporting ATPase (ATP13A2) | CSF | Immunoassay | Predictive | | | | | \$ |
| | Serum | | Predictive | | | | | |
| | Whole Blood | | Predictive | | | | | |
| Cathepsins (B, D, F, L, S) | CSF | Mass Spectrometry | Predictive | | | | | |
| | | | Susceptibility/Risk | | | | | |
| Cathepsin B activity | PBMCs | Flow Cytometry | Predictive | | | | | - |
| | | | Pharmacodynamic | | | | | |
| Cathepsin D | Plasma (brain-derived EVs) | Immunoassay | Predictive | | | | | \$ |
| Cathepsin K | CSF | Immunoassay | Predictive | | | | | \$ |
| | Serum | | Predictive | | | | | |
| | Whole Blood | | Predictive | | | | | |
| Galectin-3 | Plasma (brain-derived EVs) | Immunoassay | Predictive | | | | | \$ |
| Glucocerebrosidase (GCase, protein levels) | CSF | Mass Spectrometry | Diagnostic | | | | | \$ |
| | | | Susceptibility/Risk | | | | | |
| | Immunoassay | Predictive | | | | | | - |
| | | Pharmacodynamic | | | | | Qualified | |
| | Plasma | Immunoassay | Pharmacodynamic | | | | | Qualified |
| Plasma (brain-derived EVs) | Immunoassay | Predictive | | | | | \$ | |



= Development unsuccessful



= Samples Provided



= MJFF Funding



= Consortia

GBA & ENDOLYSOSOMAL FUNCTION CONT.

| Biomarker | Matrix | Assay Modality | Intended Use | Stage | | | | MJFF Role |
|--------------------------------------------------|------------------|-----------------------------|------------------------------|-------------|--------------|-----------------------|------------------------|-----------|
| | | | | Development | Optimization | Analytical Validation | Clinical Qualification | |
| Glucocerebrosidase activity | CSF | Mass Spectrometry | Diagnostic | | | | | \$ |
| | Dried Blood Spot | Mass Spectrometry | Prognostic | | | | | \$ |
| | | | Monitoring | | | | | \$ |
| | | | Pharmacodynamic | | | | | - |
| | Monocytes | Flow Cytometry | Prognostic | | | | | \$ |
| | | | Monitoring | | | | | |
| | | | Pharmacodynamic | | | | | |
| | PBMCs | Flow Cytometry ² | Prognostic | | | | | \$ |
| | | | Predictive ³ | | | | | \$ |
| | | | Monitoring ³ | | | | | |
| | | | Pharmacodynamic ³ | | | | | |
| | Plasma | Fluorimetric | Predictive | | | | | |
| | | | Susceptibility/Risk | | | | | |
| | Whole blood | Flow Cytometry | Predictive | | | | | - |
| Pharmacodynamic | | | | | | | | |
| Glucocerebrosidase activity (lysosomal specific) | PBMCs | Flow Cytometry ⁵ | Diagnostic | | | | | \$ |
| | | Predictive | | | | | | |
| | | Monitoring | | | | | | |

¹Prognostic and monitoring not confirmed in idiopathic PD, results from previous studies were not reproducible.












²Assay utilizes PFB-FDGlu probe.

³Longitudinal study, clinical trial of 3 repurposed drugs.

⁴GCase activity substantially lowered in GBA carriers homozygous with Gaucher's Disease. This assay is a quantitative fluorometric enzyme activity biochemical assay using 4-methylumbelliferone labeling.

⁵Assay utilizes new probe under development to detect only lysosomal-specific activity.

GBA & ENDOLYSOSOMAL FUNCTION CONT.

| Biomarker | Matrix | Assay Modality | Intended Use | Stage | | | | MJFF Role |
|------------------------------------------------------------|---------------------|-------------------|---------------------|-------------|--------------|-----------------------|----------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------|
| | | | | Development | Optimization | Analytical Validation | Clinical Qualification | |
| Transmembrane glycoprotein NMB (GPNMB) | CSF | Immunoassay | Diagnostic | | | | Did Not Qualify ¹ | \$  |
| | | | Predictive | | | | Qualified ¹ | |
| | | Mass Spectrometry | Predictive | | | | |  |
| | | | Monitoring | | | | |  |
| | Plasma | Immunoassay | Diagnostic | | | | Did Not Qualify ¹ | \$  |
| | | | Predictive | | | | Qualified ¹ | |
| Lamp-1 | CSF | Mass Spectrometry | Predictive | | | |  | |
| | | | Susceptibility/Risk | | | | | |
| Lamp-2 | CSF | Mass Spectrometry | Predictive | | | |  | |
| | | | Susceptibility/Risk | | | | | |
| Microtubule-associated proteins 1A/1B light chain 3B (LC3) | CSF | Mass Spectrometry | Predictive | | | |  | |
| Lysosomal panel ² | CSF | Immunoassay | Predictive | | | | \$ | |
| Polyamines | Plasma | Mass Spectrometry | Predictive | | | | \$  | |
| | CSF | | Predictive | | | | |  |
| Progranulin | CSF | Immunoassay | Predictive | | | | Did Not Qualify ³ |  |
| | | | Monitoring | | | | Did Not Qualify ³ | |
| | | | Prognostic | | | | Did Not Qualify ³ | |
| | Plasma ⁴ | | Pharmacodynamic | | | | | - |
| Ubiquitin | CSF | Mass Spectrometry | Predictive | | | |  | |

¹GPNMB emerged as a top hit from MJFF supported genomic, SomaLogic, O-link, and targeted lysosomal mass spectrometry efforts. Orthogonal validation in plasma using an immunoassay did not demonstrate differences between PD and controls in BioFIND, but GPNMB does have utility for subtyping & patient stratification based on an endolysosomal PD signature.

²Panel includes: p62/SQSTM1, SEC62, RTN3, ATL1, SYNGR3, SV2A, AKAP11, PKA-R1

³No differences in progranulin levels were observed in idiopathic PD, LRRK2, or GBA carriers.

⁴Progranulin levels in plasma were elevated/decreased in subjects with polymorphisms in SORT1 (rs646776) and GRN (rs5848), but no significant differences were observed in idiopathic PD, LRRK2, or GBA carriers.

LIPID HOMEOSTASIS

| Biomarker | Matrix | Assay Modality | Intended Use | Stage | | | | MJFF Role | | | |
|----------------------------------------------|---------------------------------------|-------------------|--------------------|------------------------|--------------|-----------------------|------------------------|-----------|------|------------------------------|------|
| | | | | Development | Optimization | Analytical Validation | Clinical Qualification | | | | |
| Bis(monoacylglycerol)-phosphate (BMP) | CSF | Mass Spectrometry | Prognostic | | | | | | | | |
| | | | Predictive | | | | | | | | |
| | | | Monitoring | | | | | | | | |
| | | | Pharmacodynamic | | | | Qualified ¹ | | | | |
| | Whole blood, plasma, other cell types | | Prognostic | | | | | | | | |
| | | | Predictive | | | | | | | | |
| | | | Monitoring | | | | | | | | |
| | | | Pharmacodynamic | | | | | | | | |
| | | | Urine ³ | Prognostic | | | | | | Did Not Qualify ² | |
| | | | | Predictive | | | | | | Qualified ² | |
| | | | | Monitoring | | | | | | | |
| | | | | Diagnostic | | | | | | | |
| | | | | Susceptibility/Risk | | | | | | | |
| Pharmacodynamic | | | | Qualified ¹ | - | | | | | | |
| Cholesterol derivative - Glucosylcholesterol | Urine | Mass Spectrometry | Diagnostic | | | | | | | | |
| | | | Prognostic | | | | | | | | |
| | | | Predictive | | | | | | | | |
| | | | Monitoring | | | | | | | | |

¹Responsive to LRRK2 inhibitors but less robust than urine.

²Urine BMP levels are higher in G2019S carriers independent of PD status but levels do not change with progression nor do they associate with clinical outcomes or DAT deficit. Differences only in di-22:6 BMP, not in di-18:1 BMP (for LRRK2 carriers).

³Assessment in Asian cohorts to assess BMP in carriers of other LRRK2 variants ongoing.



= Development unsuccessful



= Samples Provided




= MJFF Funding



= Consortia

LIPID HOMEOSTASIS CONT.

| Biomarker | Matrix | Assay Modality | Intended Use | Stage | | | | MJFF Role |
|-----------------------------|--------|-------------------|---------------------|-------------|--------------|-----------------------|------------------------------|----------------------------------------------------------------------------------------|
| | | | | Development | Optimization | Analytical Validation | Clinical Qualification | |
| Galactosylceramide (GalCer) | CSF | Mass Spectrometry | Diagnostic | | | | Did Not Qualify ¹ | \$  |
| | | | Prognostic | | | | Did Not Qualify ¹ | |
| | | | Susceptibility/Risk | | | | Did Not Qualify ¹ | |
| | | | Monitoring | | | | Did Not Qualify ¹ | |
| | Plasma | | Diagnostic | | | | Did Not Qualify ¹ | |
| | | | Prognostic | | | | Did Not Qualify ¹ | |
| | | | Susceptibility/Risk | | | | Did Not Qualify ¹ | |
| | | | Monitoring | | | | Did Not Qualify ¹ | |
| | Serum | | Diagnostic | | | | | |
| | | | Prognostic | | | | | |
| | | | Susceptibility/Risk | | | | | |
| | | | Monitoring | | | | | |

¹No differences observed between PD and controls, no association with any clinical outcomes or changes over time (for both PPMI and LCC cohorts).

LIPID HOMEOSTASIS CONT.

| Biomarker | Matrix | Assay Modality | Intended Use | Stage | | | | MJFF Role |
|---------------------------------------------------|--------|-------------------|---------------------|-------------|--------------|-----------------------|------------------------------|-----------|
| | | | | Development | Optimization | Analytical Validation | Clinical Qualification | |
| Glucosylceramide (GluCer, GlcCer) ¹ | CSF | Mass Spectrometry | Diagnostic | ██████████ | ██████████ | ██████████ | ██████████ | \$ 🧪 |
| | | | Prognostic | ██████████ | ██████████ | ██████████ | ██████████ | |
| | | | Susceptibility/Risk | ██████████ | ██████████ | ██████████ | ██████████ | |
| | | | Monitoring | ██████████ | ██████████ | ██████████ | ██████████ | |
| | | | Pharmacodynamic | ██████████ | ██████████ | ██████████ | Qualified ² | |
| | Plasma | | Diagnostic | ██████████ | ██████████ | ██████████ | ██████████ | \$ 🧪 |
| | | | Prognostic | ██████████ | ██████████ | ██████████ | ██████████ | |
| | | | Susceptibility/Risk | ██████████ | ██████████ | ██████████ | ██████████ | |
| | | | Monitoring | ██████████ | ██████████ | ██████████ | ██████████ | |
| | | | Pharmacodynamic | ██████████ | ██████████ | ██████████ | Qualified ² | |
| | Urine | | Diagnostic | ██████████ | ██████████ | ██████████ | ██████████ | \$ |
| | | | Prognostic | ██████████ | ██████████ | ██████████ | ██████████ | |
| Susceptibility/Risk | | ██████████ | ██████████ | ██████████ | ██████████ | | | |
| Monitoring | | ██████████ | ██████████ | ██████████ | ██████████ | | | |
| Glucosylceramide : Sphingomyelin Ratio | CSF | Mass Spectrometry | Diagnostic | ██████████ | ██████████ | ██████████ | Did Not Qualify ³ | \$ 🧪 |
| | | | Prognostic | ██████████ | ██████████ | ██████████ | Qualified ⁴ | |
| | | | Susceptibility/Risk | ██████████ | ██████████ | ██████████ | Did Not Qualify ³ | |
| | | | Monitoring | ██████████ | ██████████ | ██████████ | Qualified ⁴ | |

¹Additional analysis ongoing, but thus far did not qualify in either Schwarzschild's PPMI study or Sanofi's Venglustat trial.

²Used to show target engagement in Sanofi's Venglustat trial.

³Main differences were linked to cognitive decline in a longitudinal analysis, no clear differences between PD and controls

⁴Idiopathic PD with the highest GluCer/sphingomyelin ratios at enrollment had an accelerated longitudinal cognitive decline based on model-predicted mean Montreal Cognitive Assessment (MoCA) score.

LIPID HOMEOSTASIS CONT.

| Biomarker | Matrix | Assay Modality | Intended Use | Stage | | | | MJFF Role | |
|--------------------------------|-------------|-------------------|----------------------------------|-------------|--------------|-----------------------|------------------------|------------------------------|-----------|
| | | | | Development | Optimization | Analytical Validation | Clinical Qualification | | |
| Glucosylsphingosine (Lyso-Gb1) | CSF | Mass Spectrometry | Prognostic | ██████████ | ██████████ | ██████████ | ██████████ | 🧪 | |
| | | | Susceptibility/Risk | ██████████ | ██████████ | ██████████ | ██████████ | | |
| | | | Monitoring | ██████████ | ██████████ | ██████████ | ██████████ | | |
| | | | Pharmacodynamic | ██████████ | ██████████ | ██████████ | ██████████ | | |
| | Plasma | | Diagnostic | ██████████ | ██████████ | ██████████ | ██████████ | Did Not Qualify ¹ | 🧪 |
| | | | Susceptibility/Risk ² | ██████████ | ██████████ | ██████████ | ██████████ | | |
| | | | Monitoring ² | ██████████ | ██████████ | ██████████ | ██████████ | | |
| | | | Pharmacodynamic | ██████████ | ██████████ | ██████████ | ██████████ | | |
| | Serum | | Diagnostic | ██████████ | ██████████ | ██████████ | ██████████ | 🧪 | |
| | | | Prognostic | ██████████ | ██████████ | ██████████ | ██████████ | | |
| | | | Susceptibility/Risk ² | ██████████ | ██████████ | ██████████ | ██████████ | | |
| | Dried Blood | | Monitoring ² | ██████████ | ██████████ | ██████████ | ██████████ | - | |
| | | | Predictive | ██████████ | ██████████ | ██████████ | ██████████ | | Qualified |
| | Urine | | Diagnostic | ██████████ | ██████████ | ██████████ | ██████████ | \$ | |
| | | | Prognostic | ██████████ | ██████████ | ██████████ | ██████████ | | |
| Susceptibility/Risk | | ██████████ | ██████████ | ██████████ | ██████████ | | | | |
| Monitoring | | ██████████ | ██████████ | ██████████ | ██████████ | | | | |

¹High levels in GBA carriers regardless of PD status (BioFIND), not elevated in LRRK2 carriers or in idiopathic PD (LCC cohort). Levels were an order of magnitude higher in PD with Gaucher's disease (Alcalay). Plasma levels did not correlate with CSF levels.

²Levels thus far associate with the severity of the GBA variant. More prodromal and phenoconversion data needed to further evaluate this marker for risk and monitoring uses.

LRRK2 & ASSOCIATED EFFECTORS

Overview

The most common genetic cause of familial and sporadic PD is mutation of leucine-rich repeat kinase 2 (LRRK2). The pathophysiological mechanism behind LRRK2-PD is purported to be a toxic gain-of-function mechanism of increased kinase activity and thus the LRRK2 therapeutics at various stages of clinical development are primarily aimed at inhibiting LRRK2's aberrant kinase function or reducing production of the LRRK2 protein itself. In support of these, MJFF has deployed considerable resources to develop tools (antibodies, purified protein) and assays for phosphorylated LRRK2 and its substrates (primarily Rab GTPases). The [LRRK2 Investigative Therapeutics Exchange](#) (LITE) has a biomarkers arm that is focused on testing LRRK2 relevant biomarkers to determine if they can serve as enrichment tools for future LRRK2 trials. Given the role of LRRK2 in a variety of cellular processes which are known to be dysfunctional in PD (endolysosomal function, inflammation, etc), these pathway biomarkers may also prove relevant in evaluating LRRK2 function.

MJFF Perspective & Unmet Need

Two critical unmet needs in support of LRRK2 therapeutics include LRRK2 target engagement biomarkers in the central nervous system and enrichment biomarkers (to inform patient selection of sporadic PD subjects that could benefit from LRRK2 treatment and identify carriers who will eventually develop PD). Success to date has been hampered by the availability of antibodies, particularly for phosphorylated LRRK2, and assay platforms that are sensitive enough to detect minute levels of these proteins in relevant biofluids, particularly CSF. MJFF continues to prioritize assay development for the most common autophosphorylation site of LRRK2, pS1292, as new reagents and technologies become available, but the field is also now focusing on targeted pRab parallel reaction monitoring via mass spectrometry and pRab immunoassays. Assays for further downstream targets and processes emerging from mechanistic understanding of LRRK2's role in PD pathogenesis are also being explored (including lysosomal dysfunction, microtubule association, cilia dysfunction, etc and are covered in other sections of this report).



LRRK2 & ASSOCIATED EFFECTORS

| Biomarker | Matrix | Assay Modality | Intended Use | Stage | | | | MJFF Role |
|-----------------|--------|-------------------|--------------------------|----------------------------------|--------------|-----------------------|------------------------|-----------|
| | | | | Development | Optimization | Analytical Validation | Clinical Qualification | |
| Total LRRK2 | CSF | Mass Spectrometry | Predictive | Partially Qualified ¹ | | | \$ | |
| | | | Susceptibility/Risk | Partially Qualified ¹ | | | | |
| | | | Monitoring | Partially Qualified ¹ | | | | |
| | | | Pharmacodynamic | Qualified | | | | |
| | CSF | Immunoassay | Predictive | | | | | |
| | | | Monitoring | | | | | |
| | | | Pharmacodynamic | | | | | |
| | Serum | Immunoassay | Predictive | | | | | |
| | | | Monitoring | | | | | |
| | Plasma | Immunoassay | Predictive | Development unsuccessful | | | | |
| Pharmacodynamic | | | Development unsuccessful | | | | | |

¹LRRK2 PD have ~2x higher total LRRK2 levels in LCC samples. PPMI study confirms this effect in both rare & common variants. Analysis of longitudinal data (including idiopathic PD) in PPMI is ongoing.



= Development unsuccessful



= Samples Provided





















= MJFF Funding



= Consortia

LRRK2 & ASSOCIATED EFFECTORS CONT.

| Biomarker | Target | Assay Modality | Intended Use | Stage | | | | MJFF Role |
|-------------------------------|-------------------------|-------------------|---------------------|---------------------------------------------------------------------------------------|--------------|-----------------------|------------------------|-----------|
| | | | | Development | Optimization | Analytical Validation | Clinical Qualification | |
| Phosphorylated LRRK2 (pS935) | CSF | Immunoassay | Predictive |  | | | | \$ |
| | | | Pharmacodynamic |  | | | | |
| | Plasma | | Predictive |  | | | | |
| | | | Pharmacodynamic |  | | | | |
| | PBMCs | | Predictive |  | | | | |
| | | | Pharmacodynamic |  | | | Qualified | |
| | Whole Blood | | Pharmacodynamic |  | | | Qualified | |
| Phosphorylated LRRK2 (pS1292) | CSF | Immunoassay | Predictive |  | | | | \$ |
| | | | Susceptibility/Risk |  | | | | |
| | | | Pharmacodynamic |  | | | | |
| | Neutrophils | | Predictive |  | | | | |
| | | | Susceptibility/Risk |  | | | | |
| | | | Pharmacodynamic |  | | | | |
| | PBMCs | | Predictive |  | | | | |
| | | | Susceptibility/Risk |  | | | | |
| | | | Pharmacodynamic |  | | | | |
| Rab PRM ¹ | Monocytes & Neutrophils | Mass Spectrometry | Prognostic |  | | | | \$ |
| | | | Pharmacodynamic |  | | | | |

¹Rabs on this panel include Rab1, Rab3, Rab8, Rab10, Rab 12, Rab35 and Rab43.

LRRK2 & ASSOCIATED EFFECTORS CONT.

| Biomarker | Matrix | Assay Modality | Intended Use | Stage | | | | MJFF Role |
|---------------------|--------|----------------|---------------------|-------------|--------------|-----------------------|------------------------|-----------|
| | | | | Development | Optimization | Analytical Validation | Clinical Qualification | |
| Total Rab10 | CSF | Immunoassay | Predictive | | | | | \$ |
| | | | Susceptibility/Risk | | | | | |
| | | | Monitoring | | | | | |
| | | | Pharmacodynamic | | | | | |
| | Serum | | Predictive | | | | | |
| | | | Susceptibility/Risk | | | | | |
| | | | Monitoring | | | | | |
| | | | Pharmacodynamic | | | | | |
| | PBMCs | | Predictive | | | | | \$ |
| | | | Susceptibility/Risk | | | | | |
| | | | Pharmacodynamic | | | | | |
| | Plasma | | Predictive | | | | | |
| Susceptibility/Risk | | | | | | | | |
| Pharmacodynamic | | | | | | | | |

LRRK2 & ASSOCIATED EFFECTORS CONT.













| Biomarker | Matrix | Assay Modality | Intended Use | Stage | | | | MJFF Role |
|-------------------------------|--------|-------------------|-------------------------|-------------|--------------|-----------------------|------------------------|-----------|
| | | | | Development | Optimization | Analytical Validation | Clinical Qualification | |
| Phosphorylated Rab10 (pRab10) | Urine | Mass Spectrometry | Predictive ¹ | | | | | \$ |
| | | | Pharmacodynamic | | | | | |
| | CSF | Immunoassay | Predictive | | | | | |
| | | | Susceptibility/Risk | | | | | |
| | | | Monitoring | | | | | |
| | | | Pharmacodynamic | | | | | |
| | Serum | Immunoassay | Predictive | | | | | |
| | | | Susceptibility/Risk | | | | | |
| | | | Monitoring | | | | | |
| | | | Pharmacodynamic | | | | | |
| | Plasma | Immunoassay | Predictive | | | | | |
| | | | Susceptibility/Risk | | | | | |
| | | | Pharmacodynamic | | | | | |
| | PBMCs | Immunoassay | Predictive | | | | | |
| | | | Susceptibility/Risk | | | | | |
| | | | Pharmacodynamic | | | | | |

¹Higher pRab10 in mutation carriers demonstrated in a single cohort. Data from validation cohort is pending and will dictate whether assay should also be optimized for plasma and CSF.

²Qualification data presented via poster at Movement Disorder Society meeting in 2024 and via talk at the Alzheimer's & Parkinson's Diseases Conference in 2025.

³Used successfully in Phase 1 clinical trials.

LRRK2 & ASSOCIATED EFFECTORS CONT.

| Biomarker | Matrix | Assay Modality | Intended Use | Stage | | | | MJFF Role | |
|-------------------------------|--------|----------------|---------------------|-------------------------------------------------------------------------------------|--------------|-----------------------|------------------------|-----------|--|
| | | | | Development | Optimization | Analytical Validation | Clinical Qualification | | |
| Total Rab12 | CSF | Immunoassay | Predictive |  | | | | \$ | |
| | | | Susceptibility/Risk |  | | | | | |
| | | | Pharmacodynamic |  | | | | | |
| Phosphorylated Rab12 (pRab12) | CSF | Immunoassay | Predictive |  | | | | \$ | |
| | | | Susceptibility/Risk |  | | | | | |
| | | | Pharmacodynamic |  | | | | | |
| | Plasma | | Predictive |  | | | | | |
| | | | Susceptibility/Risk |  | | | | | |
| | | | Pharmacodynamic |  | | | | | |
| | PBMCs | | Predictive |  | | | | | |
| | | | Susceptibility/Risk |  | | | | | |
| | | | Pharmacodynamic |  | | | | | |

LRRK2 & ASSOCIATED EFFECTORS CONT.

| Biomarker | Matrix | Assay Modality | Intended Use | Stage | | | | MJFF Role |
|-------------------------------|--------|----------------|---------------------|-------------|--------------|-----------------------|------------------------|-----------|
| | | | | Development | Optimization | Analytical Validation | Clinical Qualification | |
| Total Rab29 | CSF | Immunoassay | Predictive | | | | | \$ |
| | | | Susceptibility/Risk | | | | | |
| | | | Pharmacodynamic | | | | | |
| | Plasma | | Predictive | | | | | |
| | | | Susceptibility/Risk | | | | | |
| | | | Pharmacodynamic | | | | | |
| | PBMCs | | Predictive | | | | | |
| | | | Susceptibility/Risk | | | | | |
| | | | Pharmacodynamic | | | | | |
| Phosphorylated Rab29 (pRab29) | CSF | Immunoassay | Predictive | | | | | \$ |
| | | | Susceptibility/Risk | | | | | |
| | | | Pharmacodynamic | | | | | |
| | Plasma | | Predictive | | | | | |
| | | | Susceptibility/Risk | | | | | |
| | | | Pharmacodynamic | | | | | |
| | PBMCs | | Predictive | | | | | |
| | | | Susceptibility/Risk | | | | | |
| | | | Pharmacodynamic | | | | | |

INFLAMMATION & IMMUNE DYSFUNCTION

Overview

Immune function has been linked to PD in numerous ways: postmortem examination of PD brains shows increased inflammation & infiltration of adaptive immune cells, genetic studies have linked immune genes like HLA-DR with PD risk, & chronic use of anti-inflammatory compounds like NSAIDs & TNF inhibitors have been linked to lower PD risk. Chronic immune activation has also been linked to alpha-synuclein aggregation & increased mitochondrial dysfunction which exacerbate disease progression. The challenge, however, has been identifying biomarkers to detect this immune dysfunction in accessible biofluids or tissues. Immune activation has been investigated in PD via soluble markers using readily available panels in blood and CSF, with varying results depending on the assay platform, cohort, or biofluid evaluated. To help resolve these variations, the field is shifting toward cellular immunophenotyping, which has provided evidence for dysfunction in both the innate and adaptive immunity in PD, including shifts in subpopulations of monocytes, T cells & B cells. More recently, findings related to inflammasome pathway markers have sparked interest, as these directly relate to inflammatory related cell death. This interest is reflected in the increasing number of immune therapies being pursued in clinical trials. Importantly, while immune biomarkers have potential prognostic, stratification and target engagement applications, their potential as standalone diagnostic markers is limited due to the range of neurological conditions involving chronic immune dysfunction, despite the historical focus on their diagnostic potential in the literature.

MJFF Perspective & Unmet Need

Soluble immune mediators are complex because 1) these markers are expressed at low levels necessitating highly reliable & sensitive assays, 2) primary PD risk factor is aging, & immune function is influenced by concurrent conditions prevalent in the aging community, 3) majority of cytokines are affected by diurnal fluctuations & transient activation making consistency of detection a critical & complex issue. Cellular changes are more consistent with less variability; however, these results still have inconsistencies across studies in part due to protocol variation and differences in markers across panels. To this end, recent efforts have been focused on bringing the community together to establish methodology recommendations for sample handling, storage and protocol standardization. Ultimately, the aim of this Immune Consortium is to help identify robust protein or cell-based immune signatures in PD, independently validate findings across different labs using complementary methods, and lay the groundwork to establish a time course for immune changes in longitudinal PD samples. A longitudinal time course is critical to better understand how these immune pathways become dysfunctional over the course of the disease and how quickly patients may respond to immunomodulatory therapies. In addition to those markers tracked here, many discovery studies are ongoing that assess immune function in PD (e.g. cellular immunophenotyping, autoantibody panels), and outcomes of these projects will likely yield additional markers to include in future iterations of this report.



INFLAMMATION & IMMUNE DYSFUNCTION

| Biomarker | Matrix | Assay Modality | Intended Use | Stage | | | | MJFF Role |
|------------------------------------------------------------------------------|-----------|----------------|-----------------|-------------|--------------|-----------------------|------------------------|-----------|
| | | | | Development | Optimization | Analytical Validation | Clinical Qualification | |
| Apoptosis-associated speck-like protein containing a CARD (ASC) ¹ | CSF | Immunoassay | Monitoring | | | | | \$ |
| | Plasma | | Diagnostic | | | | | |
| Caspase-1 ¹ | CSF | Immunoassay | Predictive | | | | | \$ |
| | Plasma | | Diagnostic | | | | | |
| Endophenotype (pro-inflammatory) | Plasma | Immunoassay | Diagnostic | | | | | \$ |
| | | | Predictive | | | | | |
| Fibrinogen ³ | CSF | Immunoassay | Diagnostic | | | | | - |
| | | | Prognostic | | | | | |
| Glial fibrillary acid protein (GFAP) | CSF | Immunoassay | Prognostic | | | | | \$ |
| | | | Predictive | | | | | |
| | | | Monitoring | | | | | |
| Gasdermin D (GSDMD) ¹ | CSF | Immunoassay | Predictive | | | | | \$ |
| | Plasma | | Predictive | | | | | |
| High mobility group box 1 (HMGB1) | CSF | Immunoassay | Monitoring | | | | | \$ |
| | Plasma | | Predictive | | | | | |
| Heme oxygenase 1 (HMOX1) | Monocytes | Immunoassay | Pharmacodynamic | | | | | \$ |

¹Important components of NLRP3 inflammasome pathway activation

²Proteomic multi-marker algorithm successfully distinguished PD from controls in a preliminary study using a 2-step screening method: first to distinguish the signature of neurodegenerative disease from controls, second to distinguish PD from other neurodegenerative diseases. This study utilized proteomic assays on the MSD platform for 20 markers: A2M, B2M, CRP, eotaxin-3, FABP-3, Factor 7, I-309, ICAM1, IL-5, IL-6, IL-7, IL-10, IL-18, pancreatic polypeptide, serum amyloid A, TARC, tenascin C, thrombopoietin, TNF- α , and VCAM1. Replication in additional cohort(s) required.

³Increased in blood with high inflammation and may be mediated via astrocytes. Upregulated in PD with cognitive impairment (detected by both RNA and ELISA protein levels). Replication needed in larger multi-site cohort.

⁴Marker for astrocytes. No significant differences in PPMI between PD and controls or longitudinally. May be useful when combined with other markers or in subtyping efforts.



= Development unsuccessful



= Samples Provided















= MJFF Funding



= Consortia

INFLAMMATION & IMMUNE DYSFUNCTION CONT.

| Biomarker | Matrix | Assay Modality | Intended Use | Stage | | | | MJFF Role |
|-----------------------------------------------------------------------------------------------|-----------|----------------------------|-------------------------|--------------------------------------------------------------------------------------|--------------|-----------------------|------------------------|----------------------------------------------------------------------------------------|
| | | | | Development | Optimization | Analytical Validation | Clinical Qualification | |
| Immunoglobulin G (IgG) index | CSF | Immunoassay | Prognostic | <i>Partially Qualified¹</i> | | | | \$  |
| Matrix metalloproteinase 10 (MMP-10)² | CSF | Immunoassay | Monitoring | | | | | - |
| Neutrophil Lymphocyte Ratio (NLR) | Blood | Complete blood count (CBC) | Diagnostic | <i>Partially Qualified³</i> | | | | - |
| | | | Prognostic ⁴ | | | | | |
| Nuclear factor kappa-light-chain-enhancer of activated B cells subunit p65 (NF-kB p65) | Monocytes | Immunoassay | Pharmacodynamic |  | | | | \$ |
| NLR family pyrin domain containing 3 (NLRP3)⁵ | CSF | Immunoassay | Predictive |  | | | | \$  |
| | | | Monitoring |  | | | | |
| | Plasma | | Predictive |  | | | | |
| | | | Monitoring |  | | | | |
| Autoreactive T cells (PINK1 & aSyn)⁶ | PBMCs | Stimulation Assay | Prognostic |  | | | | \$  |
| Toll-like receptor 2 (TLR2) | Monocytes | Immunoassay | Pharmacodynamic |  | | | | \$ |
| Toll-like receptor 4 (TLR4) | B cells | Flow Cytometry | Diagnostic |  | | | | - |
| | Monocytes | | Diagnostic |  | | | | |

¹Baseline index used as potential indicator or future cognitive decline. Independent replication needed in larger, multi-site cohort.

²Correlates with disease progression in external study. Replication needed in larger, multi-site cohort.

³NLR has been shown to be higher in idiopathic PD and GBA PD compared to controls (although NLR in LRRK2 PD was comparable to controls) and higher in those at risk for PD compared to controls. Importantly, NLR elevation is not specific to PD and thus cannot be used as a standalone diagnostic tool.

⁴Small pilot studies have shown association of high NLR with lower striatal DAT levels and association of higher NLR with faster cognitive decline measured via MoCA. These findings should be replicated in larger cohorts.

⁵Important component of NLRP3 inflammasome activation

⁶Autoimmune response used to detect upregulated inflammation in PD, T cells specifically reactive to both PINK1 and aSyn

INFLAMMATION & IMMUNE DYSFUNCTION CONT.

| Biomarker | Matrix | Assay Modality | Intended Use | Stage | | | | MJFF Role |
|---------------------------------------------|--------|---------------------------------------------------------|---------------------|-------------|--------------|-----------------------|------------------------|-----------|
| | | | | Development | Optimization | Analytical Validation | Clinical Qualification | |
| Various cytokines & chemokines ¹ | CSF | Mass Spectrometry/ Stimulation Assay/ Immunoassay | Diagnostic | | | | Discordant findings | \$ ⓘ |
| | | | Prognostic | | | | Discordant findings | |
| | | | Predictive | | | | Discordant findings | |
| | | | Monitoring | | | | Discordant findings | |
| | | | Pharmacodynamic | | | | Discordant findings | |
| | Plasma | | Diagnostic | | | | Discordant findings | |
| | | | Prognostic | | | | Discordant findings | |
| | | | Predictive | | | | Discordant findings | |
| | | | Susceptibility/Risk | | | | Discordant findings | |
| | | | Monitoring | | | | Discordant findings | |
| | Serum | | Diagnostic | | | | Discordant findings | |
| | | | Prognostic | | | | Discordant findings | |
| | | | Monitoring | | | | Discordant findings | |

¹Various studies conducted on a wide variety of markers yielded inconsistent results, though overall, results indicate elevation of pro-inflammatory markers in PD. More work is needed with sensitive, reliable proteomic assays to establish pro-inflammatory phenotypes and potential prognostic value of these signatures in PD. Further, assays specific to the active/cleaved form for these markers may be more reliable than assays measuring total levels of the analyte. Assessment in isolated cell types with more sensitive assays incorporating independent validation with complementary methods will better establish immune signatures for PD and disease stages at which these signatures are most relevant. Lead cytokine/chemokine candidates based on multiple meta-analyses are CCL5, CRP, IL-1B, IL-2, IL-6, IL-10, IL-18 and TNF-a.

MITOCHONDRIAL DYSFUNCTION

Overview

Many aspects of mitochondrial function including reactive oxygen species production, mitophagy, mitochondrial biogenesis, ion homeostasis, and mitochondrial trafficking are being pursued as potential therapeutic targets in both genetic and sporadic Parkinson's disease (PD). Furthermore, epidemiological studies indicate that chronic exposure to environmental toxins that disrupt mitochondrial complex I is associated with an increased risk of developing PD and may play a major role in disease pathogenesis. Biomarkers that can specifically reveal a pathological exposure to toxicants would help identify individuals in the prodromal phase of the disease who could benefit from early pharmacological interventions. Overall, validation and qualification of mitochondrial biomarkers will enable better stratification (of both genetic and idiopathic patients) to support therapeutic trials targeting this pathway and be more broadly applicable to PD disease progression.

MJFF Perspective & Unmet Need

Measuring cellular organelle functionality in biofluids presents unique challenges since tissue-specific dysfunction might not reflect pathology in the brain, and relevant analytes are difficult to detect in CSF. Additionally, analytes related to oxidative stress, while detectable in fluids, require specific and rigorous sample collection methods to preserve the oxidation state after collection, as the oxidation state is critical to fully understand the extent of dysregulation for these pathways in PD. Thus far, the most advanced mitochondrial dysfunction biomarker efforts have focused on mitophagy (due to the functional role of genetically-linked genes, Parkin and PINK1, in removal of damaged mitochondria). However, mitochondrial dysfunction is the nexus of many pathways and processes that are disrupted in PD, and it is therefore critical to expand biomarker work beyond these genetic targets and mitophagy endpoints. One avenue that may hold promise is to evaluate markers from other relevant fields such as primary mitochondrial disease in PD cohorts. Future efforts should continue to focus on understanding the full picture of mitochondrial dysfunction in relevant matrices at multiple disease stages.



MITOCHONDRIAL DYSFUNCTION

| Biomarker | Matrix | Assay Modality | Intended Use | Stage | | | | MJFF Role |
|-----------------------------------------------------------------------------------------------------------|--------|----------------|-----------------|-------------|--------------|-----------------------|------------------------|-----------|
| | | | | Development | Optimization | Analytical Validation | Clinical Qualification | |
| Protein deglycase DJ-1 (DJ-1, PARK7) | CSF | Immunoassay | Diagnostic | | | | | \$ |
| | | | Predictive | | | | | |
| | Plasma | | Diagnostic | | | | | |
| | | | Predictive | | | | | |
| DJ-1, PARK7 redox states (reduced, oxidized, superoxidized) | Plasma | Immunoassay | Diagnostic | | | | | \$ |
| | | | Predictive | | | | | |
| G protein-coupled receptor 37 ecto domain (ecto-GPR37, parkin-associated endothelin-like (Pael) receptor) | CSF | Immunoassay | Diagnostic | | | | | \$ |
| | | | Prognostic | | | | | |
| | Plasma | | Diagnostic | | | | | |
| Growth differentiation factor 15 (GDF15, also MIC-1 or pTGFB) | Plasma | Immunoassay | Diagnostic | | | | | \$ |
| | | | Predictive | | | | | |
| Mitochondrial Rho GTPase 1 (Miro1) ¹ | PBMCs | Immunoassay | Diagnostic | | | | | \$ |
| | | | Pharmacodynamic | | | | | |

¹PD difference observed but only in response to stressor, limiting potential utility in a biofluid panel. Not a circulating marker & must be measured in cells.



= Development unsuccessful



= Samples Provided



= MJFF Funding



= Consortia



MITOCHONDRIAL DYSFUNCTION CONT.

| Biomarker | Matrix | Assay Modality | Intended Use | Stage | | | | MJFF Role |
|--------------------------------------------|------------|------------------|-----------------|-------------|--------------|-----------------------|------------------------|-----------|
| | | | | Development | Optimization | Analytical Validation | Clinical Qualification | |
| mitoPD ¹ | PBMCs | Functional Assay | Predictive | | | | | \$ |
| | Muscle | | | | | | | |
| mtDNA copy number, transcription, deletion | CSF | Genetic (RT-PCR) | Diagnostic | | | | | \$ |
| | | | Predictive | | | | | |
| | Serum | | Diagnostic | | | | | |
| | | | Predictive | | | | | |
| mtDNA damage | Buffy coat | Genetic (PCR) | Diagnostic | | | | | \$ |
| | | | Predictive | | | | | |
| | | | Pharmacodynamic | | | | | |
| | PBMCs | | Diagnostic | | | | | |
| | | | Predictive | | | | | |
| Parkin | Plasma | Immunoassay | Predictive | | | | | \$ |
| Parkin (PS65) | Plasma | Immunoassay | Predictive | | | | | \$ |
| PTEN-induced putative kinase 1 (PINK1) | Plasma | Immunoassay | Predictive | | | | | \$ |

¹This line item describes a mitochondrial subtype of idiopathic PD, and is comprised of quantitative and functional assessments for each mitochondrial respiratory chain complex, as well as evaluating mtDNA copy number and deletion fraction assessment.

²Thus far, data have shown higher mtDNA damage in idiopathic PD compared to controls and in LRRK2 PD, which can be reversed with inhibitors. Independent replication needed, as well as additional efforts to translate this assay into more easily accessible matrices.

MITOCHONDRIAL DYSFUNCTION CONT.

| Biomarker | Matrix | Assay Modality | Intended Use | Stage | | | | MJFF Role |
|------------------------------------------|-------------|-------------------|---------------------|------------------------|--------------|-----------------------|------------------------------|----------------------------------------------------------------------------------------|
| | | | | Development | Optimization | Analytical Validation | Clinical Qualification | |
| Pyridoxamine 5'-phosphate oxidase (PNPO) | Dried Blood | Mass Spectrometry | Diagnostic | | | | | \$ |
| | | | Predictive | | | | | |
| Urate | CSF | Mass Spectrometry | Prognostic | | | | Qualified ¹ | \$  |
| | | | Pharmacodynamic | | | | Qualified ¹ | |
| | Plasma | | Prognostic | | | | Qualified ¹ | |
| | | | Susceptibility/Risk | | | | Qualified ¹ | |
| | | | Pharmacodynamic | | | | Qualified ¹ | |
| | Serum | | Monitoring | | | | Did Not Qualify ¹ | |
| | | | Prognostic | | | | Qualified ¹ | |
| Pharmacodynamic | | | | Qualified ¹ | | | | |
| Phosphorylated ubiquitin (pS65Ub) | CSF | Immunoassay | Diagnostic | | | | Did Not Qualify ² | \$  |
| | | | Monitoring | | | | Qualified ² | |
| | Plasma | | Diagnostic | | | | Did Not Qualify ² | |
| | | | Monitoring | | | | Qualified ² | |

¹Urate in plasma, CSF, and serum is an inverse risk factor for idiopathic PD. Higher urate levels are linked to lower PD incidence across multiple natural history and clinical trial studies and levels correlate across matrices. Data from LCC demonstrated that non-manifesting LRRK2 mutation carriers had significantly higher levels of urate than those who developed PD, substantiating urate as a biomarker of resistance to PD among LRRK2 mutation carriers. In serum from PPMI, urate concentrations were stable over 5 years and did not correlate with worsening measures of PD progression suggesting that serum urate is not a monitoring biomarker of PD, at least in early stages

²Modest changes over time in PD using commercially available assay at Millipore. Assay does not show consistent changes across matrices when comparing PD to controls. Potential for predictive and/or pharmacodynamic uses have not yet been evaluated.

NEURODEGENERATION & NEUROPROTECTION

Overview






Biomarkers of neurodegeneration, alone, are not specific for Parkinson's disease (PD) diagnosis. Nonetheless, analytes that reflect protein pathology, dopamine dysfunction and axonal degeneration may be relevant in combination with more disease-specific markers, useful in predicting cognitive outcomes in PD, pertinent for differential diagnosis from related neurodegenerative disorders, and helpful in denoting disease progression. Most of these assays were initially developed and analytically validated for use in the Alzheimer's field, where there is both good correlation between peripheral and central compartments as well as orthogonal validation with imaging approaches.

MJFF Perspective & Unmet Need

The PD field should continue to piggyback off of well-characterized, analytically-validated neurodegeneration assays where strong rationale for evaluating a deeply-phenotyped PD cohort exists. Modelling approaches leveraging these broad markers of neurodegeneration combined with disease-specific markers may be useful in subtyping and will support the evolving Neuronal Synuclein Disease Integrated Staging System.



NEURODEGENERATION & NEUROPROTECTION

| Biomarker | Matrix | Assay Modality | Intended Use | Stage | | | | MJFF Role |
|---------------------------|-------------------|-----------------------------------------------|-------------------------|-------------|--------------|-----------------------|------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| | | | | Development | Optimization | Analytical Validation | Clinical Qualification | |
| Apolipoprotein A1 (ApoA1) | Plasma | Immunoassay | Prognostic | | | | Qualified ¹ |  |
| | Serum | | Diagnostic | | | | | - |
| | | | Prognostic | | | | Qualified | - |
| Apolipoprotein E (ApoE4) | DNA (Whole Blood) | Biochemical/ Genetic (Allele Specific Probes) | Prognostic | | | | Did Not Qualify ² |  |
| Amyloid-beta 1-42 (Aβ42) | CSF | Immunoassay | Diagnostic | | | | Did Not Qualify ³ |   |
| | | | Prognostic | | | | Qualified ⁴ | |
| | | | Monitoring ⁵ | | | | Qualified ⁴ | |
| Catecholamines | CSF | Mass Spectrometry | Diagnostic | | | | Did Not Qualify ⁶ |  |
| | | | Monitoring | | | | Did Not Qualify ⁶ | |
| Cortisol | Plasma | Immunoassay | Prognostic | | | | Qualified | - |

¹Lower ApoA1 is correlated with earlier PD onset (multiple cohorts, including PPMI). In the Parkinson's Associated Risk Study (PARS) cohort, lower levels were associated with higher DAT deficit. Changes not very robust.

²Although ApoE4 genotype has been associated with more rapid cognitive decline in other cohorts, the results did not replicate in PPMI. Since ApoE4 genotype is significantly associated with CSF Aβ42, that may more directly mediate cognitive dysfunction in PD.

³CSF Aβ42 levels were lower in PD than controls (reduction in PD levels at baseline is not diagnostically meaningful).

⁴Neurodegeneration biomarker predicts cognitive decline.

⁵CSF Aβ42 levels decrease over time, which is consistent with β-amyloid pathology.

⁶Minimal changes observed between PD and controls in some analytes; no change over one year in unmedicated PD.



= Development unsuccessful



= Samples Provided



= MJFF Funding



= Consortia

NEURODEGENERATION & NEUROPROTECTION CONT.

| Biomarker | Matrix | Assay Modality | Intended Use | Stage | | | | MJFF Role |
|--------------------------------------|-------------------|----------------|--------------|------------------------------|--------------|-----------------------|------------------------|-----------|
| | | | | Development | Optimization | Analytical Validation | Clinical Qualification | |
| DOPAdecarboxylase (DDC) | CSF | Immunoassay | Prognostic | | | | | \$ |
| | | | Predictive | | | | | |
| | | | Monitoring | | | | | |
| | Plasma | | Prognostic | | | | | |
| | | | Predictive | | | | | |
| | | | Monitoring | | | | | |
| | HPLC enzyme assay | Predictive | | | | | | |
| | | Monitoring | | | | | | |
| Epidermal growth factor (EGF) | Plasma | Immunoassay | Prognostic | Did Not Qualify ¹ | | | | |
| Insulin-like growth factor-1 (IGF-1) | Serum | Immunoassay | Diagnostic | Did Not Qualify ² | | | | \$ |
| | | | Prognostic | Qualified ³ | | | | |
| Klotho | CSF | Immunoassay | Diagnostic | | | | | - |
| | | | Prognostic | | | | | |

¹Inconsistent findings across cohorts. Low baseline EGF levels in external cohorts correlate with future cognitive decline or low performance in visuospatial tests, but validation in PPMI was unsuccessful.

²In contrast to early findings in small pilot cohorts, no significant differences observed between PD & controls in PPMI cohort, indicating it is not a diagnostic marker

³Low IGF-1 correlates to poor executive task performance & predicts cognitive deficits. High IGF-1 correlates with higher UPDRS-3.















NEURODEGENERATION & NEUROPROTECTION CONT.

| Biomarker | Matrix | Assay Modality | Intended Use | Stage | | | | MJFF Role |
|--------------------------------------------|--------|----------------|---------------------|---------------------------|--------------|-----------------------|------------------------|-----------|
| | | | | Development | Optimization | Analytical Validation | Clinical Qualification | |
| Neurofilament-light (NfL) ¹ | CSF | Immunoassay | Diagnostic | Did Not Qualify | | | | \$ 🧪 |
| | | | Monitoring | Qualified | | | | |
| | Serum | | Diagnostic | Qualified | | | | 🧪 |
| | | | Monitoring | Qualified | | | | |
| | Plasma | | Prognostic | | | | | \$ 🧪 |
| | | | Susceptibility/Risk | | | | | |
| | | | Monitoring | | | | | |
| Neurogranin | CSF | Immunoassay | Diagnostic | Inconclusive ² | | | | \$ 🧪 |
| | | | Monitoring | Inconclusive ² | | | | |
| Neuronal pentraxins (NPTX1, NPTX2, NPTXR) | CSF | Immunoassay | Diagnostic | | | | | - |
| | | | Prognostic | | | | | |
| | | | Monitoring | | | | | |
| | Plasma | | Diagnostic | | | | | |
| | | | Prognostic | | | | | |
| | | | Monitoring | | | | | |
| Nuclear preceptor-related factor 1 (NURR1) | PBMCs | RT-PCR | Diagnostic | | | | | \$ |

¹In CSF, different cohorts/assays yielded different results and may be underpowered. Serum data are more clear: NfL is the first biochemical biomarker to increase over time in PD (albeit very slowly), and it correlates with motor and cognitive measures. Given that NfL levels are highest in other neurodegenerative disorders compared to PD, it may be a useful tool in differentiating between synucleinopathies. Combined modelling with other PD-relevant biomarkers is needed. Ongoing proteomic efforts in PPMI plasma include evaluation of prodromal and genetic cohorts.

²Inconclusive results in PPMI. Consider replicating in expanded PPMI/prodromal cohorts.

NEURODEGENERATION & NEUROPROTECTION CONT.

| Biomarker | Matrix | Assay Modality | Intended Use | Stage | | | | MJFF Role |
|----------------------------------------------------------------|--------|----------------|---------------------|---------------------------------------------------------------------------------------|--------------|-----------------------|------------------------|----------------------------------------------------------------------------------------|
| | | | | Development | Optimization | Analytical Validation | Clinical Qualification | |
| Total tau | CSF | Immunoassay | Diagnostic | Did Not Qualify ¹ | | | | \$  |
| | | | Prognostic | Qualified ² | | | | |
| | | | Monitoring | Did Not Qualify ³ | | | | |
| Phosphorylated tau (p-tau 181) | CSF | Immunoassay | Diagnostic | Did Not Qualify ¹ | | | | \$  |
| | | | Prognostic | Qualified ² | | | | |
| | | | Monitoring | Did Not Qualify ³ | | | | |
| Phosphorylated tau (pTau-217) | Plasma | Immunoassay | Diagnostic |  | | | |  |
| | | | Prognostic |  | | | | |
| | | | Monitoring |  | | | | |
| Phosphorylated tau (pTau-231) | CSF | Immunoassay | Diagnostic |  | | | | - |
| | | | Prognostic |  | | | | |
| | | | Monitoring |  | | | | |
| eMTBR-243 (tau fragment containing microtubule-binding region) | CSF | Immunoassay | Diagnostic |  | | | | - |
| | | | Prognostic |  | | | | |
| | | | Monitoring |  | | | | |
| Vitamin D (calciferol) | Plasma | Immunoassay | Susceptibility/Risk |  | | | | - |
| | Serum | | Prognostic |  | | | | |

¹Reduction in PD levels at baseline is not diagnostically meaningful.

²Core Alzheimer's biomarkers predict cognitive decline.

³CSF p-tau (181) and total tau showed no change over 5-year follow-up. Well-validated assays for other p-tau species are available and should be examined in PD.

ADDITIONAL MARKERS

Overview

The previous sections have highlighted major pathways relevant to PD, but there are additional markers that might be relevant to PD biology and pathogenesis that fall outside these buckets. In order to make this report inclusive of the PD field as a whole, additional markers not covered in the previous sections have been included here.

MJFF Perspective & Unmet Need

MJFF and the field at large are actively pursuing biomarkers that could support PD diagnosis, monitoring, target engagement, etc, regardless of whether they fall into high priority target categories or are related to biological pathways most thought to be dysfunctional in PD. As with many of the previous sections, it is vital to understand whether the following markers can be useful toward refining disease staging and/or identifying subsets of patients that might benefit from specific therapies.



ADDITIONAL MARKERS

| Biomarker | Matrix | Assay Modality | Intended Use | Stage | | | | MJFF Role |
|------------------------------------------------|--------|----------------|-------------------------|-------------|--------------|-----------------------|------------------------|-----------|
| | | | | Development | Optimization | Analytical Validation | Clinical Qualification | |
| <i>Cell adhesion</i> | | | | | | | | |
| Vascular cell adhesion molecule (VCAM1) | Serum | Immunoassay | Prognostic | | | | | - |
| <i>Circadian</i> | | | | | | | | |
| Melatonin | Plasma | Immunoassay | Diagnostic | | | | | |
| | | | Prognostic | | | | | |
| | Serum | | Diagnostic ¹ | | | | | |
| | | | Prognostic | | | | | - |
| <i>DNA Repair</i> | | | | | | | | |
| Poly-ADP ribose (PAR) | CSF | Immunoassay | Diagnostic | | | | | |
| | | | Prognostic | | | | | |
| | | | Monitoring | | | | | |

¹No significant differences observed in MJFF's 24-Hour Biofluid cohort

²PAR in CSF does not predict diagnosis, cognitive, or motor decline in multiple longitudinal PD cohorts, including PPMI



= Development unsuccessful



= Samples Provided



= MJFF Funding



= Consortia

ADDITIONAL MARKERS

| Biomarker | Matrix | Assay Modality | Intended Use | Stage | | | | MJFF Role |
|-----------------------------------------------------------------------------|--------|-----------------------------------|--------------|------------------------------|--------------|-----------------------|------------------------|-----------|
| | | | | Development | Optimization | Analytical Validation | Clinical Qualification | |
| <i>Insulin Regulation</i> | | | | | | | | |
| Amylin | Plasma | Immunoassay | Diagnostic | | | | | - |
| | | | Prognostic | | | | | |
| | | | Monitoring | | | | | |
| <i>Metabolism</i> | | | | | | | | |
| VGF (central metabolic regulator, highly inducible via nerve growth factor) | CSF | Mass Spectrometry | Diagnostic | | | | | \$ |
| | | | Prognostic | | | | | |
| Vitamin B12 (cobalamin) | CSF | Mass Spectrometry/ Immunoassay | Prognostic | Did Not Qualify ¹ | | | | \$ |
| | | | Monitoring | Did Not Qualify ¹ | | | | |
| | Plasma | | Prognostic | Did Not Qualify ¹ | | | | |
| | | | Predictive | Did Not Qualify ¹ | | | | |
| | | | Monitoring | Did Not Qualify ¹ | | | | |

¹Although preliminary data in small PD cohorts were promising, findings did not replicate in larger cohorts.



APPENDIX



