PARKINSON'S DISEASE TARGET REPORT

March 2023

Report Objectives and Overview

This report provides a brief overview of targets of interest from MJFF's portfolio. Profiles for emerging and advanced targets provide a summary of the current status of the target as it relates to therapeutic development, how it is connected to Parkinson's Disease, and what MJFF's investments have been up to this point. Novel targets at an earlier exploratory stage are listed in a table as the available information may be limited. As targets advance from novel to emerging, and remain of interest, profiles will be built out to contain additional details.

This report is not comprehensive of every potential target being explored today but represents those **recently supported by MJFF** that have been identified by research staff as important to monitor and build awareness around. There are additional advanced clinical targets within the MJFF portfolio that are not included here but can be found in MJFF's *Clinical Pipeline Report* and *Biomarker Report*. Although not currently in scope, there is the possibility of expanding to targets outside of MJFF's portfolio in future iterations.

Statements and categorizations made throughout may be subjective and based on MJFF expertise and opinion. All information included in this report is publicly available and is intended to be used as a resource for researchers and investors evaluating opportunities and/or seeking new projects.

> **Target Status Definitions:**
>
> **Novel:** *A target that has been previously reported to have a link to Parkinson's disease through genetics/expression or a shared cellular pathway with other neurodegenerative diseases*
>
> **Emerging:** *A target that has a stronger link to Parkinson's disease through genetics/expression/known cellular pathways AND additional data on target modulation (through genetics or pharmacological manipulation) in PD relevant models AND/OR some interest from stakeholders at academia/industry/CROs*
>
> **Advanced:** *A target that has strong links to Parkinson's disease through genetics/expression/known cellular pathways AND target modulation data in endogenous PD models AND sufficient interest from stakeholders at academia/industry/CROs*
>
> *While target status often correlates with stage of drug development, above definitions are tied to the strength of the target's connection to PD*

The report will be fully updated annually with individual target profiles reviewed on a rolling basis.

For any comments or suggestions, please contact:
- Shalini Padmanabhan (spadmanabhan@michaeljfox.org)
- Andrew Koemeter-Cox (akoemetercox@michaeljfox.org)

MJFF INITIATIVES

MJFF continuously evaluates the landscape for opportunities to invest in initiatives that will de-risk targets and enable the entire research community. The active programs below will be highlighted throughout the report on relevant target profiles.

BRIDGE INITIATIVE

Co-led by Drs. Paul Galatsis and Darren Moore, the Bridge Initiative aims to develop field-enabling tool compounds against key genetic and functional targets of Parkinson's Disease. The Bridge Initiative will use state-of-the-art medicinal chemistry techniques, such as DNA-encoded library screening (DEL), and functional assays, to identify small molecule binders to important targets in PD. The three main criteria to choose the targets to be pursued have been 1) Genetic link to PD, 2) Functional relevance in PD development, 3) Lack of available tool compounds. Within the wide array of possible targets, and based on the above-described criteria, the Bridge Initiative Steering Committee has selected GBA1 and Parkin as starting targets. Experiments are expected to kick off in 2023 and will run for 1-2 years. If successful, the Initiative will not only provide useful chemical matter but will also pave the way for future efforts in developing further publicly available, field-enabling tool compounds against the most promising therapeutic targets in Parkinson's disease as novel targets arise.

TOOLS

The research community continues to develop new research streams as new genetic/genomic targets and mechanistic pathways are linked to Parkinson's disease. To support these new research streams and enable broader early-stage investigation, vetting of potential targets, and continued work on established targets, MJFF works with the research field to identify gaps in the research tools space and fill those gaps by generating new tools or transferring tools from labs to open access repositories. MJFF currently makes available many tools for advanced targets and is developing additional tools for novel, emerging, and advanced targets.

To learn more about our tools program, please visit https://www.michaeljfox.org/research-tools. Additionally, you can find a complete list of the tools MJFF has made available through our program and the additional tools in our development pipeline through our Research Tools Catalog at https://www.michaeljfox.org/research-tools-catalog.

The Michael J. Fox Foundation for Parkinson's Research

REPORT OVERVIEW

TARGET STATUS

43% of included emerging targets have a genetic link to PD
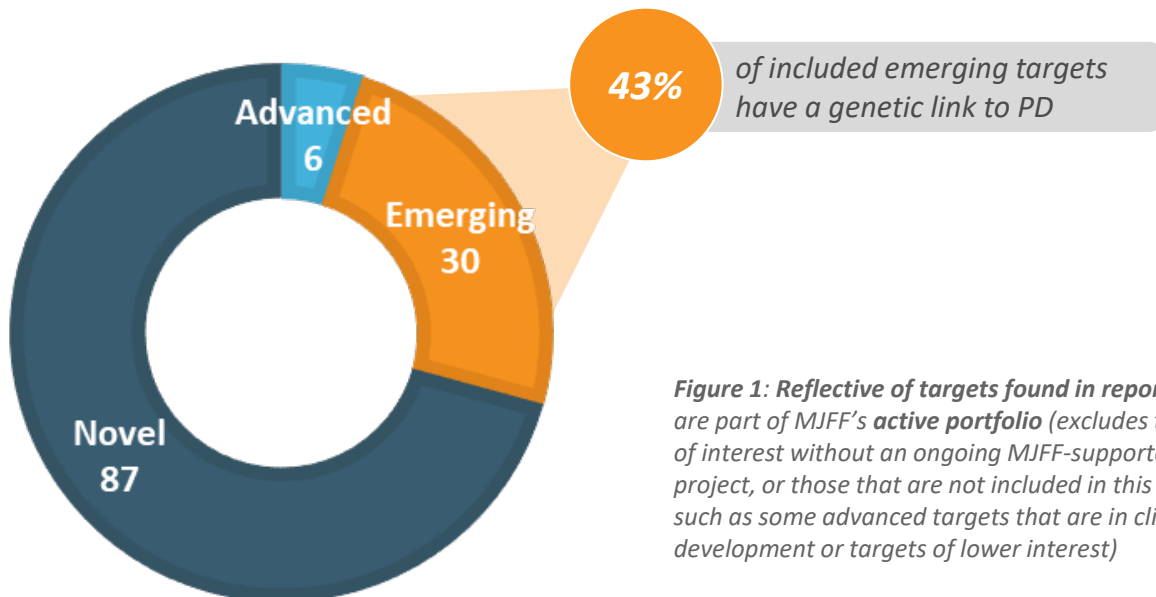
Advanced 6
Emerging 30
Novel 87

*Figure 1: **Reflective of targets found in report** that are part of MJFF's **active portfolio** (excludes targets of interest without an ongoing MJFF-supported project, or those that are not included in this report, such as some advanced targets that are in clinical development or targets of lower interest)*

TARGETS BY PATHWAY

Inflammation 28
Other (n≤2) 18
Mitochondria 14
Alpha-Synuclein Spread 13
Lysosome 12
Ub/Cellular degradation 11
Endolysosome 10
Symptomatic 10
Oxidative Stress 6
Autophagy 5
Stress response pathway 5
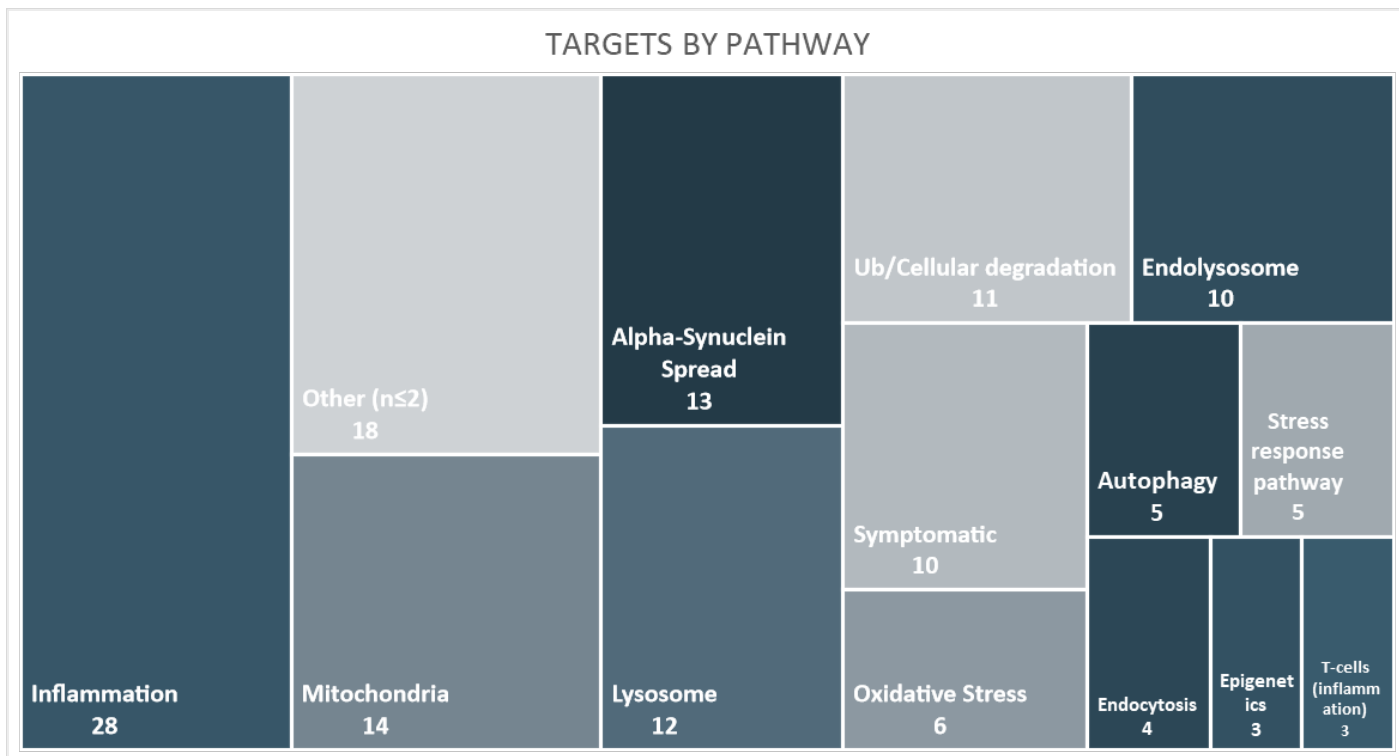Endocytosis 4
Epigenetics 3
T-cells (inflammation) 3

*Figure 2: **Reflective of targets found in report** that are part of MJFF's **active portfolio**. Targets with multiple pathways are counted for each (e.g., a target associated with inflammation and lysosome will be included in the count for both pathways). "Other" includes pathways that have only 1 target linked (e.g., cell adhesion). n = 126*

SAMPLE TARGET PROFILE

(Full Target Name)

Novel > Emerging > Advanced

Primary Pathway(s)

Overview

- *Connection to PD*: (Genetics/Pathway/Expression)
- *Non-PD indications: Other disease indications linked to gene/protein and/or have a potential therapeutic intervention currently being developed (highest development status noted)*
- *Modulation approaches: Target modulation approaches actively available (commercial) or in therapeutic development*

What's Needed?

- *From MJFF's perspective, what is needed next to take this forward as a field? What resources are needed? What questions still need to be answered? MJFF may be working to address one or more of these (refer to MJFF Perspective section).*

Target Summary

Scientific overview of target and how it is relevant to Parkinson's Disease, including a brief summary of relevant research that been completed up to this point (field-wide and MJFF-supported projects).

MJFF Perspective

A summary of MJFF activities and next steps for this target. Depending on the target and amount of information available, some or all the following may be highlighted:
- The potential impact of ongoing projects
- Opportunities and/or bottlenecks for future development
- What questions should be prioritized

MJFF Supported Projects	*Active PD Companies*
- All past and current MJFF funded projects related to target (year of funding)	- All companies actively working on or supporting therapeutic development against this target for PD. Non-PD companies are included if they have a relevant platform or are not yet indication specific (e.g. neuroinflammation) but have explicitly noted the potential and/or interest to develop for PD

NOVEL TARGETS

A target that has been previously reported to have a link to Parkinson's disease through genetics/expression or a shared cellular pathway with other neurodegenerative diseases

NOVEL TARGETS

The below table highlights novel targets of interest that are currently, or have recently been, a part of MJFF's portfolio. As targets move through development and generate additional data, full target profiles will be built out.

Target	Protein/Target Name	Pathway	Genetic Target
22q11.2 locus			Y
5AR1	5-alpha reductase type 1	Symptomatic	N
AAK1	Adapter associated kinase 1	Endocytosis	Y (weak)
ASAH1	N-acylsphingosine amidohydrolase 1	Lysosome	Y
ATP10B	ATPase phospholipid transporting 10B	Endolysosome	Y
C5AR1	Complement C5a receptor 1	Inflammation	N
CACNA1D	Voltage dependent L-type calcium channel subunit alpha-1D	Oxidative Stress	N
CCR2	C-C motif chemokine receptor 2	Inflammation	Y (weak)
CHCHD2	Coiled-coil-helix-coiled-coil-helix domain containing 2	Mitochondria	Y
CHMP2B	Charged multivesicular body protein 2b	Endosomal sorting	N
CIITA	class II major histocompatibility complex transactivator	Inflammation	N
CLR	C-type Lectin receptor	Inflammation	N
CORO1C	Coronin-1C	Cytoskeletal organization	Y
CRAC	calcium release activated channel	Inflammation	N
CSF1R	Colony Stimulating Factor 1 Receptor	Inflammation	N
Cx32/GJB1	Gap junction protein beta 1	Alpha-Synuclein Spread	N
DCC	Deleted in Colorectal Cancer/netrin 1 receptor	Stress response pathway	N
DNAJC6	Auxilin	Endocytosis	Y
DRD3	dopamine receptor D3	Inflammation	N
FAIM	Fas apoptotic inhibitory molecule 1	Ub/Cellular degradation	N
FASN	Fatty acid synthase	Mitochondria	N
FCGR2B (FCGRIIB)	Fc fragment of IgG receptor IIb	Inflammation	N
FXR/NR1H4	Nuclear receptor subfamily 1 group H member 4	Inflammation	N
GALC	Galactosylceramidase	Lysosome	Y
GBAP1	Glucosylceramidase beta pseudogene 1	Lysosome	Y
GDF15	Growth differentiation factor 15	Stress response pathway	N

NOVEL TARGETS

Target	Protein/Target Name	Pathway	Genetic Target
GLYT1/SLC6A9	Sodium- and chloride-dependent glycine transporter 1	Symptomatic	N
GM-CSF	Granulocyte-macrophage colony-stimulating factor	Inflammation, T-cells	N
GPR37	Prosaposin receptor GPR37	Ub/Cellular degradation	N
HDAC4	Histone deacetylase 4	Epigenetics	N
HSD17B3	Hydroxysteroid 17-beta dehydrogenase 3	Symptomatic	N
I2BS	Imidazoline-2 binding site	Inflammation	N
INPP5F	Inositol polyphosphate-5-phosphatase F	Endocytosis	Y
KAT8/KANSL1	Lysine acetyltransferase 8/KAT8 regulatory NSL complex subunit 1	Mitochondria	Y
LAMP2A	Lysosome associated membrane protein	Autophagy/Lysosome	N
LIMP2/SCARB2	Scavenger receptor class B member 2	Lysosome	Y
LRP1	Low-density lipoprotein receptor-related protein 1	Cell Adhesion	N
LUBAC	Linear ubiquitin chain assembly complex	Ub/Cellular degradation	N
Miro	Miro like atypical Rho GTPases	Mitochondria	Y (weak)
MLK3/MAP3K11	Mitogen-activated protein kinase kinase kinase 11	Inflammation	N
MLKL	Mixed lineage kinase domain like pseudokinase	Stress response pathway	N
mtDNA	Mitochondrial DNA	mitochondrial DNA	N/A
N/A	Labile Iron	Oxidative Stress	N
N/A	Mitochondrial complex I	Mitochondria	N
mPTP	Mitochondrial permeability transition pore	Inflammation/Mitochondria	N
Ndufaf2	NADH:ubiquinone oxidoreductase complex assembly factor 2	Mitochondria	N
Nedd4	NEDD4 E3 ubiquitin protein ligase	Ub/Cellular degradation	N
Neurexin1ß	Neurexin 1	Alpha-Synuclein Spread	N
NFE2L1	nuclear factor, erythroid 2 like 1	Oxidative Stress	N
OGA	O-GlcNAcase	Alpha-Synuclein Spread	N
PAK6	p21 (RAC1) activated kinase 6	Endolysosome pathway/LRRK2	N

NOVEL TARGETS

Target	Protein/Target Name	Pathway	Genetic Target
PARIS/ZNF746	Zinc finger protein 746	Mitochondria	N
PER1	period circadian regulator 1	Symptomatic (sleep)	N
PGK1	Phosphoglycerate Kinase 1	Mitochondria	N
PIKFYVE	Phosphatidylinositol 3-phosphate 5-kinase	Lysosome	N
PKA RII(α/β)	Protein kinase A regulatory subunits IIα and IIβ	Symptomatic	N
PPM1H	Protein phosphatase, Mg2+/Mn2+ dependent 1H	LRRK2	N
pRab10	phosphoRab10	Endolysosome, alpha-synuclein spread	N
RILPL1/2	Rab interacting lysosomal protein like 1/2	Ciliogenesis	N
RIP140/NRIP1	nuclear receptor interacting protein 1	Mitochondria	N
ROCK	Rho-associated protein kinase	Inflammation	N
S2R	Sigma-2 receptor	Symptomatic	N
Saposin-C/PSAP	Prosaposin	Lysosome	N
SLC39A9	N/A	Alpha-Synuclein Spread	N
SCARB2	Lysosome membrane protein 2	Lysosome	Y
SMPD1	Sphingomyelin phosphodiesterase 1	Autophagy/Lysosome	Y
Sod1	Superoxide dismutase 1	Oxidative Stress	Y
SynIII	Synapsin III	Alpha-Synuclein Spread	N
SYNJ1	Synaptojanin-1	Endocytosis	Y
TET2	Methylcytosine dioxygenase TET2	Epigenetics	N
TGM2	Transglutaminase 2	Alpha-Synuclein spread	N
TLR9	toll like receptor 9	Inflammation	Y
TM9SF2	transmembrane 9 superfamily member 2	Alpha-Synuclein Spread	N
TMA	Trimethylamine (metabolite, not a protein)	Inflammation	N
TNFR2	TNF receptor	Inflammation	N
TRIMs	Tripartite motif containing proteins	Ub/Cellular degradation	Y
USP14	Ubiquitin specific peptidase 14	Ub/Cellular degradation	N
USP8	Ubiquitin carboxyl-terminal hydrolase 8	Ub/Cellular degradation	N
VPAC2	vasoactive intestinal peptide receptor 2	Inflammation, T-cells	N
XBP-1	X-box binding protein 1	Stress response pathway	N

The Michael J. Fox Foundation for Parkinson's Research

EMERGING TARGET PROFILES

A target that has a stronger link to Parkinson's disease through genetics/expression/known cellular pathways AND additional data on target modulation (through genetics or pharmacological manipulation) in PD relevant models AND/OR some interest from stakeholders at academia/industry/CROs

- ✓ **5-HT1A**
- ✓ **ABL1/C-ABL**
- ✓ **ATP13A2**
- ✓ **CB2/CNR2**
- ✓ **CK1δ/ε**
- ✓ **CTSB**
- ✓ *DRD1*
- ✓ *GLP-1*
- ✓ **GPNMB**

- ✓ **GPR43**
- ✓ *MAP3K11 (MLK3)*
- ✓ **MAPT**
- ✓ **Neuromelanin (NM)**
- ✓ **Nurr1 /NR4A2**
- ✓ **PARK7/DJ-1**
- ✓ **PARP1**
- ✓ **PGRN**
- ✓ **Rabs (Rab29/Rab8b)**

- ✓ **RIPK1**
- ✓ **SCD1&5**
- ✓ **STING1**
- ✓ **TMEM175**
- ✓ **TRPML1/MCOLN1**
- ✓ **USP30**
- ✓ **VPS13C**
- ✓ **VPS35**

✓ Target portfolio included

5-HT1A

(Serotonin 1A receptor)

Novel	**Emerging**	Advanced

Pathway: symptomatic

Overview

5-HT1A is a target that has the potential to treat LIDs
- *Connection to PD*: symptoms
- *Non-PD indications**: Alzheimer's/Dementia (preclinical) Bipolar disorder (clinical), Cancer (preclinical), Depression (clinical), Inflammatory/ autoimmune disease (preclinical), Pain (clinical), OCD (clinical), Schizophrenia (launched)
- *Modulation approaches*: small molecule

What's Needed?

- *Additional studies with 5-HT1A modulators to confirm efficacy in the clinic*
- *More selective 5-HT1A modulators*

*Includes indications for both agonists and antagonists

Target Summary

The serotonin 1A receptor (5-HT1A) is expressed in brain regions relevant to Parkinson's disease, including the striatum. Activation of the receptor can modulate dopamine release, making the receptor a candidate for the treatment of Levodopa-Induced Dyskinesia (LID), which eventually affect nearly all Parkinson's patients during the course of their disease. While showing promise preclinically, 5-HT1A receptor agonists have shown mixed efficacy in clinical trials. Some have shown hints of efficacy in clinical trials while other 5HT1-A agonists have worsened motor performance. This has been mostly attributed to the non-selective nature of many 5-HT1A compounds.

MJFF Perspective

5HT1A is an attractive target for ameliorating dyskinesia in PD patients. MJFF is currently supporting the company Neurolixis in conducting a clinical trial with befiradol (NLX-112) that acts to reduce the amount of dopamine release from serotonergic neurons. This clinical trial will potentially validate 5HT1A as an important target for dyskinesia, mood, disturbed sleep, and pain. MJFF is also supporting a project to illuminate the circuitry of this target pathway.

MJFF Supported Projects	*Active PD Companies* *($: direct or indirect funding given)*
- Lund University (2008, 2009, 2010) - Molecular Neuroimaging (2011) - Neurolixis (2011, 2013, 2014, 2014, 2020) - PsychoGenics (2010) - State University of New York (2011, 2012, 2013, 2020) - University of Cagliari (2013)	$ Neurolixis (Phase 2) - Sumitomo Dainippon (Phase 1)

ABL1/C-ABL

(Tyrosine-protein kinase ABL1)

Novel → **Emerging** → Advanced   🗑 *Pathway: autophagy*

Overview

c-Abl1 inhibition in the brain may offer a neuroprotective treatment for patients with PD and related disorders.

- *Connection to PD*: pathway
- *Non-PD indications*: Oncology (launched), MSA (preclinical), LBD (preclinical)
- *Modulation approaches*: small molecule

What's Needed?

- *Novel approaches to target this pathway*
- *Validation of aberrant activation of c-Abl in PD*
- *Better biomarkers to measure the analyte in the CNS*

Target Summary

c-Abl is a nonreceptor tyrosine kinase that has been a successful drug target for chronic myelogenous leukemia. There have been reports that inhibiting c-Abl may ameliorate Parkinson's disease symptoms and other alpha-synucleinopathies. Recently, there have been multiple clinical trials attempting to re-purpose the tyrosine kinase inhibitor Nilotinib in Parkinson's patients. However, these studies have not been able to demonstrate target engagement and thus, there is still no clinical interventional data demonstrating a role of c-abl in Parkinson's patients.

MJFF Perspective

While there is still an interest in targeting c-Abl to prevent disease progression in Parkinson's patients, the field is in need of a potent and selective c-Abl inhibitor that can cross the blood brain barrier and c-Abl in the brain. MJFF has supported other potential therapeutics to target c-Abl and assay development to optimize target engagement readouts in the clinic.

MJFF Supported Projects	Active PD Companies ($: direct or indirect funding given)
• Brigham & Women's Hospital (2017, 2020)	• 1st Biotherapeutics (Phase 1)
• Ecole Polytechnique Federale de Lausanne (2017)	• Il-Yang Pharmaceutical (Phase 2)
• Inhibikase Therapeutics (2016, 2019)	$ Inhibikase Therapeutics (Phase 2)
• Johns Hopkins (2017)	• KeifeRx (Phase 3)
• MPI Research (2016)	• SPARC (Phase 2)
• Northwestern University (2017)	
• University of Texas Health Science Center (2007)	

ATP13A2

(Polyamine-transporting ATPase 13A2) 💉

Novel → **Emerging** → Advanced   ♻ *Pathway: endolysosome*

Overview

Candidate PD-associated gene that encodes a lysosomal P-type transport ATPase.
- *Connection to PD*: genetic
- *Non-PD indications*: Kufor-Rakeb syndrome
- *Modulation approaches*: N/A

What's Needed?

- *Further validation of ATP13A2 as a viable target for PD*
- *Novel approaches to targeting ATP13A2*
- *Generation of tools to probe target function in endogenous systems*
- *Assessment of target dysfunction in patient samples*

Target Summary

ATP13A2, a lysosomal ATPase, is mutated in autosomal recessive forms of early-onset Parkinson's disease. Loss-of-function mutations in ATP13A2 destabilize the protein and prevents lysosomal degradation of substrates and subsequent accumulation of alpha-synuclein. Another study also implicated ATP13A2 in the transport of spermine and glucosylceramide out of the lysosome thus preventing the accumulation of toxic lysosomal substrates.

MJFF Perspective

Current MJFF efforts include a HTS and hit characterization study to identify ATP13A2 agonists and further mechanistic studies to validate the role of ATP13A2 on mitophagy and alpha-synuclein pathology using patient-derived iPSCs and PD rodent models.

Future efforts will focus on using genetic and pharmacological approaches to further validate ATP13A2 as a therapeutic target for PD and assessing the levels of glucosylceramide and spermine in patient biosamples to translate our understanding of the target into developing better biomarkers for PD.

MJFF Supported Projects	Active PD Companies ($: direct or indirect funding given)
• Ecole Polytechnique Federale de Lausanne (2011) • Garvan Institute (2012) • KU Leuven (2011, 2013, 2020, 2020)	• Centre for Drug Design and Discovery (Preclinical)

CB2/CNR2

(Cannabinoid receptor 2)

Novel → **Emerging** → Advanced

Pathway: inflammation

Overview

CB2 agonists and cannabidiol (CBD) may have the potential to reduce a-syn induced inflammation in PD patients.

- *Connection to PD: expression*
- *Non-PD indications*: ALS (preclinical), Anxiety (preclinical), Autism (clinical), Autoimmune/ Inflammatory diseases (clinical), Cancer (preclinical), Dementia/AD (preclinical), Epilepsy (preclinical), HD (preclinical), MS (clinical), Muscular dystrophy (preclinical), Nausea (launched), Pain (clinical), Sleep disorders (clinical), Weight loss (launched)*
- *Modulation approaches: small molecule*

*Includes indications for both agonists and antagonists

What's Needed?

- *Preclinical evidence that CB2 modulation can reduce PD-specific inflammation*
- *More specific CB2 antibodies*
- *More human data on central and peripheral expression of CB2 in PD patients*

Target Summary

Cannabinoid receptor 2 (CB2) is mostly expressed in peripheral immune cells, where is it thought to regulate the activity of these cells. More recently, it has been determined that CB2 expression is increased on microglia in the brains of PD patients and in certain animal models of Parkinson's. CB2 is also of high interest since the cannabinoid cannabidiol (CBD) is thought to modulate the receptor.

MJFF Perspective

Since preliminary evidence indicates that CB2 is specifically upregulated in pathologic states, it has begun to emerge as an attractive target for the modulation of inflammation in a few different diseases, including Parkinson's. MJFF is funding work to determine if CB2 antagonism can reduce inflammation elicited by Parkinson's relevant stimuli. If results from these studies are positive, the development of better CB2 relevant tools, especially antibodies and centrally-penetrant compounds, will be an area of need.

MJFF Supported Projects	Active PD Companies *($: direct or indirect funding given)*
• University of Florida Board of Trustees (2020) • Emory University (2015) • Universidad Complutense (2012)	• *No known companies*

CK1δ/ε

(Casein kinase I)

Novel	**Emerging**	Advanced

Pathway: symptomatic (sleep)

Overview

CK1, a biological clock regulator, is a potential disease-modifying target to improve sleep and Circadian function among PD patients.
- *Connection to PD: symptoms*
- *Non-PD indications: Alzheimer's (preclinical), Cancer (clinical), Sleep disorder (clinical)*
- *Modulation approaches: small molecule*

What's Needed?

- *Further deep characterization of sleep dysfunction in preclinical PD models*
- *Preclinical evidence that CK1δ/ε modulation can fix sleep dysfunction in preclinical PD models*
- *Further information on sleep dysfunction in human PD*

Target Summary

Casein Kinase 1 (CSNK1A1) is involved in regulating the core biological clock, via modulation of Period2, or PER2. Both pharmacological and genetic modulation of CSNK1A1 in mice has shown the ability to control multiple aspects of sleep, and correct aberrant phenotypes in mice. It is known that sleeping patterns are disrupted in prodromal and full PD, but the pathways modulating this disruption are not still fully understood.

MJFF Perspective

Treatment for sleep fragmentation is often cited as one of the highest current unmet needs by people with Parkinson's. Currently there are few promising leads on modulating sleep in Parkinson's. MJFF is funding research to characterize sleep fragmentation in preclinical models, and test the ability of CSNK1A1 modulation to correct sleep dysfunction in preclinical models.

MJFF Supported Projects	*Active PD Companies* ($: direct or indirect funding given)
• University of Queensland (2021)	• Neumora Therapeutics (Preclinical)*

*Current development not indication specific, potential for several diseases, including PD

CTSB
(Cathepsin B) 🔧

Novel | **Emerging** | Advanced • *Pathway: lysosome*

Overview

Variants in CTSB that decreases its expression leads to increased risk in GBA-PD
- *Connection to PD*: genetic, pathway
- *Non-PD indications*: Alzheimer's (preclinical); Cancer (preclinical); Infection (preclinical); TBI (preclinical)
- *Modulation approaches*: small molecule, vaccine

What's Needed?

- *Understand the role of CTSB in lysosomal dysfunction, GCase function and synuclein aggregation in PD*
- *Further validation of the variants in altering GCase activity*
- *Better tools to manipulate expression and detect changes in CTSB function*

Target Summary

CTSB is a lysosomal protease, previously shown to be involved in α-synuclein degradation. Additionally, variants in CTSB are associated with penetrance of GBA-PD. The outcomes of the ongoing research, noted below, will inform future studies aimed at identifying the sub-population of GBA mutation carriers who are at higher risk for developing Parkinson's disease.

MJFF Perspective

Current MJFF efforts include further investigation of the involvement of CTSB in PD generally and in relation to GCase, using patient-derived iPSCs and dopaminergic neurons. Ongoing studies are also directed at fine-mapping the CTSB locus to identify the most likely variants driving the associations with PD risk and penetrance and examining whether CTSB directly interacts with GCase in the lysosome.

Future efforts would focus on developing better tools to visualize the target under endogenous conditions and/or manipulate the target in vivo to test its impact on PD phenotypes.

MJFF Supported Projects	Active PD Companies *($: direct or indirect funding given)*
• McGill University ([2018](#), [2020](#)) • *Tools Development:* ○ University of Oxford (2019)	• *No known companies*

DRD1 *(Dopamine Receptors D1, D2)*

Novel → **Emerging** → Advanced

Pathway: symptomatic

Overview

DARs are GPCRs that signal through both G-proteins and beta-arrestin (barr)
- *Connection to PD*: Receptor for Dopamine
- *Non-PD indications**: n/a
- *Modulation approaches*: Small Molecule

What's Needed?

- *More targeted approaches to dopamine system modulation that avoid side effects*
- *Better assays to assess dopamine receptor signaling*

Target Summary

Overexpression of beta-arrestin 2 (barr2) in animal models has been shown to reduce L-DOPA-induced dyskinesias in animal model. Beta-arrestin 2 is a modulator of DRD1 signaling. Early drug development programs are attempting to develop small molecule agonists to activate only barr2-dependent signaling at D1 and D2. In theory, such an agonist would retain anti-Parkinsonian effects without causing dyskinesia.

MJFF Perspective

MJFF is funding novel approaches to downstream modulation of DRD1 and DRD2 signaling to avoid the side effect profiles associated with many current PD medications. Current funding involves improvement of chemical matter that targets barr2 signaling.

MJFF Supported Projects	Active PD Companies ($: direct or indirect funding given)
- Duke University (2019) - University of Florida (2016)	- *No known companies*

GCG/GLP-1

(Glucagon-like peptide-1)

Novel | **Emerging** | Advanced

Pathway: growth factor signaling

Overview

Normalization of growth factor signaling (eg. insulin, NGF, GDNF) in the brain, normalizing energy utilization and mitochondrial function, dopamine synthesis and synaptic transmission, and a reduction of the chronic inflammation response in the CNS. Additionally, alpha-synuclein levels are reduced by normalizing autophagy.

- *Connection to PD: Pathway*
- *Non-PD indications*: n/a*
- *Modulation approaches: Analogous of the hormone*

What's Needed?

To both decipher MOA of GLP-1 analogs and to identify both pharmacodynamic and target/pathway engagement biomarkers that could be incorporated in ongoing/planned clinical trials through:

- *Preclinical studies*
- *Deep analyses of existing human data*

Target Summary

The most noteworthy effect of GLP-1 is its ability to promote insulin secretion in a glucose-dependent manner. In the brain, GLP-1 receptor activation has been linked with neurotrophic effects including neurogenesis and neuroprotective effects including reduced necrotic and apoptotic signaling, cell death, and dysfunction.

MJFF Perspective

Several trials have shown that treatment with GLP-1 analogous has a positive effect in PD patients' motor symptoms (no effect observed on non-motor or LID). However, multiple pathways seem to be involved in GLP-1 analogous' effect on PD patients, and it is difficult to demonstrate central target/pathway engagement.

MJFF Supported Projects	Active PD Companies ($: direct or indirect funding given)
• University College London (2018)	• Kariya Pharmaceuticals (Preclinical) • Neuraly (Phase 2) • Peptron (Phase 2) • Sanofi/University Hospital of Toulouse (Phase 2)

GPNMB

(Transmembrane glycoprotein NMB) 🔧

| Novel | **Emerging** | Advanced |

Pathway: inflammation & lysosome

Overview

Variants in GPNMB are associated with PD risk and elevations in GPNMB levels are observed in PD
- *Connection to PD*: expression, genetic, pathway
- *Non-PD indications*: ALS (preclinical); Amyloidosis (preclinical); Cancer (preclinical)
- *Modulation approaches*: antibody

What's Needed?

- *Understand the impact of polymorphisms on gene expression and subsequent neurodegeneration mechanisms*
- *Preclinical tools and clinical assays to quantify expression and activity of GPNMB in PD animal models and human biofluids*

Target Summary

GPNMB is a transmembrane glycoprotein that has been implicated in lipid homeostasis and immune regulation. Genetic studies have linked polymorphisms in GPNMB with risk for PD and increased expression of GPNMB is linked to disease pathogenesis. GPNMB is elevated in biofluids of mice with GBA deficiencies, in serum of patients with Gaucher disease and in SN of PD patients. However, overexpression of GPNMB has also shown to protect dopamine neurons in the MPTP model and recombinant GPNMB has shown to attenuate LPS-induced inflammation in primary mouse microglia suggesting that increasing the levels of GPNMB can be therapeutic in PD.

MJFF Perspective

Current MJFF efforts focus on assessing the levels of GPNMB in plasma from PD patients and generating preclinical tools such as plasmids and proteins to further probe the biology of GPNMB.

In the future, it would be interesting to further dissect the compensatory and beneficial effects of GPNMB in relevant neuronal and non-neuronal cells, understand the mechanisms by which GPNMB variants cause PD pathogenesis and expand on the biomarker efforts to validate GPNMB as a disease biomarker.

MJFF Supported Projects	Active PD Companies ($: direct or indirect funding given)
• University of Alabama – Birmingham (2016, 2019) • University of Oxford (2020) • *Tools Development:* o University of Oxford (2019)	• *No known companies*

GPR43

(G-protein coupled receptor 43)

Novel **Emerging** Advanced

Pathway: Inflammation, T-cells

Overview

GPR43 is a GPCR highly expressed in T-cells which is activated by pathogenic forms of a-syn in PD
- *Connection to PD*: expression, pathway
- *Non-PD indications*: chronic inflammation, obesity, asthma, arthritis, multiple sclerosis, psoriasis (all preclinical), colitis (clinical)
- *Modulation approaches*: gene therapy, small molecule

What's Needed?

- *Preclinical validation of GPR43 as a PD-relevant target for inflammation*
- *More knowledge of GPR43 activity in PD patients*
- *Further mechanistic information on GPR43 role in T-cells for PD – more relevant for the gut or CNS*

Target Summary

Short chain fatty acid (SCFA) receptor that may explain the finding that SCFAs produced by gut bacteria are a factor in PD development among susceptible individuals. Mouse models show that a-syn overexpression via the Thy1 promoter may be responsible for the activation of a-syn specific T-cells (activation of inflammatory phenotypes), leading to a downstream autoimmune response in the nigrostriatal pathway.

MJFF Perspective

While GPR43 has been explored as a potential therapeutic target for inflammatory GI conditions such as colitis, direct evidence of therapeutic potential for PD has not yet been demonstrated. MJFF is currently funding work that would provide preclinical validation of GPR43 as an inflammatory target for PD, as well as assessing the therapeutic potential for a small molecule GPR43 antagonist in preclinical PD. This compound has already demonstrated safety and tolerability in humans.

MJFF Supported Projects	Active PD Companies *($: direct or indirect funding given)*
• Fundación Ciencia & Vida (2022)	• *No known companies*

MLK3/ MAP3K11

(mixed lineage protein kinase-3/mitogen-activated protein kinase kinase kinase 11)

| Novel | **Emerging** | Advanced |

Pathway: Inflammation & apoptosis

Overview

MLK3 has been linked to regulation of apoptosis and neuroinflammation, and inhibition protects against neuron loss in PD models
- *Connection to PD*: pathway
- *Non-PD indications*: HIV-1 associated neurocognitive disorder (preclinical); NASH (preclinical); Cancer (preclinical); MS (preclinical); Cardiovascular disease (preclinical); Alzheimer's (preclinical); Stroke (preclinical); Arthritis (preclinical); Colitis (preclinical)
- *Modulation approaches*: small molecule, siRNA

What's Needed?

- *Further validation of MLK3 as therapeutic target in preclinical PD genetic models*
- *Measurement of MLK3 activity in PD patient samples*
- *More potent inhibitors of MLK3 and similar kinases that are CNS-penetrant*
- *Markers of target engagement*

Target Summary

MLK3 has been implicated in pro-apoptotic pathways shown to be upregulated in neurodegenerative diseases including PD. Specifically, MLK3 is known to activate the JNK pathway, which is upregulated in preclinical PD models. This kinase has also been identified as a critical activator in microglia and macrophages for AP-1, a transcription factor upregulating pro-inflammatory genes. These linkages suggest that MLK3 inhibition may be beneficial for both neuroprotection and reducing neuroinflammation in PD. A previous clinical trial assessing a first generation MLK3 inhibitor in PD demonstrated compound safety, but no efficacy, dampening enthusiasm for this pathway in the pharmaceutical space. However, further studies of this compound (CEP-1347) showed poor CNS penetrance, reopening the potential of MLK3 as a therapeutic target for PD.

MJFF Perspective

Previous MJFF-funded efforts indicate that MLK3 inhibition alone may be insufficient to modify PD disease course in preclinical models. One known MLK3 inhibitor is URMC-099, which also inhibits LRRK2. Because of LRRK2's known genetic link to PD, it is unclear whether the preclinical benefits observed with URMC-099 can be linked directly to MLK3 or whether they are more likely attributed to effects of the compound on LRRK2. Further studies are needed to better understand the specific role of MLK3 in PD and the interplay between MLK3 with similar kinases for relevant pathways including apoptosis and neuroinflammation.

MJFF Supported Projects	Active PD Companies *($: direct or indirect funding given)*
- University of Rochester (2018) - Califia Biosciences (2013)	- *No known companies*

MAPT
(Microtubule associated protein tau)

Novel ▸ **Emerging** ▸ Advanced

Pathway: microtubule function

Overview

Tau plays an important role in axonal transport and variants in MAPT locus linked to PD risk
- *Connection to PD: genetic*
- *Non-PD indications: Alzheimer's (clinical); Epilepsy (preclinical); FTD (clinical); MSA (clinical); PSP (clinical), TBI (clinical)*
- *Modulation approaches: small molecule*

What's Needed?

- *Developing and testing new biochemical and imaging assays for Tau/pTau in human LRRK2 PD and iPD samples*
- *Test the effect of modulating Tau levels and phosphorylation on improvement of PD relevant phenotypes*
- *Fine mapping of the locus to identify causal variants*

Target Summary

MAPT is a phosphorylated protein primarily expressed in the brain where it aids in the stabilization of the cytoskeleton and axonal transport in neurons. Variants in MAPT have shown to increase the risk of PD and influence the progression and clinical manifestations of the disease. Additionally, autopsy of PD patients have revealed the colocalization of Tau and α-Syn in Lewy bodies and several tau assays have been developed to quantify total and phosphorylated tau levels in human biosamples. More recent findings have also highlighted the incidence of tau pathology in LRRK2 mutation carriers, a finding that has prompted research on studying the impact of LRRK2 on tau aggregation, spreading and release. Therapeutic approaches include the development of a small molecule inhibitor of tau oligomerization.

MJFF Perspective

Current efforts at MJFF are focused on understanding the mechanisms by which Tau contributes to the pathophysiology of LRRK2 PD and iPD. MJFF is also funding Tau imaging studies in LRRK2 carriers and deploying tau and phospho-tau assays in the PPMI study. These studies will help identify groups at high risk for the development of PD and provide the possibility for early preventive measures.

Future studies would focus on fine mapping the locus and identifying MAPT genotypes that correlate with PD risk and progression in different ethnic populations. Future investments will also test the beneficial effect of decreasing tau levels using PD models.

MJFF Supported Projects	*Active PD Companies* *($: direct or indirect funding given)*
• Columbia University (2021) • Institute for Neurogenerative Disorders (2020) • Trustees of University of Pennsylvania (2016, 2019) • Van Andel Research Institute (2018, 2019) • Cantabio (2020, 2021) • Psy Therapeutics (2021) • *Tools Development:* ○ University of Oxford (2019)	• Alterity Therapeutics (Phase 1) $ Cantabio Pharmaceuticals (Preclinical) • Nuravax (Preclinical) $ Psy Therapeutics (Preclinical)

NM

(Neuromelanin)

| Novel | **Emerging** | Advanced |

⚡ *Pathway: oxidative stress*

Overview

Neuromelanin is the pigment that accumulates in Substantia Nigra Dopaminergic neurons and has recently been implicated as a pathogenic factor
- *Connection to PD: expression, genetics, patient samples*
- *Non-PD indications: N/A*
- *Modulation approaches: N/A*

What's Needed?

- *Completion of phenotyping of Neuromelanin-expressing animal models*
- *Completion of experiments to assess if NM modulation alters PD pathology in preclinical models*

Target Summary

Neuromelanin is the dark pigment that accumulates in dopaminergic neurons of the substantia nigra and locus coeruleus. In humans, it is thought that neuromelanin is a downstream by-product of dopamine and L-DOPA oxidation and metabolism in cells. Rodent models do not naturally accumulate neuromelanin in their dopaminergic neurons, which could be one factor in the difficulty of translating findings from these models to humans. Neuromelanin accumulates with age, and age is the leading risk factor for developing Parkinson's disease. Evidence from the Vila lab suggest that there is a "pathogenic threshold" of neuromelanin accumulation in neurons, above which cell toxicity and death occurs.

The Vila lab has also created rodent models which accumulate neuromelanin in substantia nigra dopaminergic neurons via human-tyrosinase, inserted either via a viral vector or germline transgene. The Vila lab has shown neuromelanin accumulation in these rodents, even in the absence of alpha-synuclein, can result in PD-like pathology, and that removal of neuromelanin from neurons reduces or even eliminates this pathology.

MJFF Perspective

MJFF is currently funding work to further validate the Vila lab rodent model, as well as explore factors that influence the rate of accumulation of neuromelanin. With further validation, the neuromelanin-accumulating rodent model could be another model to develop and test therapeutic strategies for Parkinson's. It is currently unclear how neuromelanin accumulation could be targeted as a viable therapeutic strategy but understanding factors that influence accumulation will certainly increase biological understanding of the disease.

MJFF Supported Projects	*Active PD Companies* *($: direct or indirect funding given)*
• Baylor College of Medicine (2011) • Emory University (2021) • MSDx (2013, 2014) • National Research Council, Italy (2003) • University of California, Riverside (2015) • Vall d'Hebron Institute of Research (2018, 2019, 2020)	• *No known companies*

NR4A2/NURR1   *(Nuclear receptor subfamily 4 group A member 2)*

Novel → **Emerging** → Advanced

Pathway: transcriptional regulation

Overview

Nurr1 is critical for the maintenance and survival of dopaminergic neurons
- *Connection to PD*: genetic
- *Non-PD indications*: Alzheimer's (preclinical); inflammatory/ autoimmune diseases (preclinical); MS (preclinical)
- *Modulation approaches*: small molecule

What's Needed?

- *Selective Nurr1 small molecule agonists*
- *Markers of target engagement*

Target Summary

Nurr1 is a transcription factor that regulates many genes critical for the development and survival of dopaminergic neurons. Specifically, Nurr1 upregulates the transcription of Tyrosine Hydroxylase (TH) and Vesicular Monoamine Transporter 2 (VMAT2), among other genes. Nurr1 also modulates neuroinflammation by repressing the transcription of genes coding for proinflammatory cytokines. Nurr1 expression is reduced in PD patients compared to aged-matched controls, and animal models deficient in Nurr1 recapitulate many PD-like behavioral and pathological features.

MJFF Perspective

A major roadblock to further development of Nurr1 as a target has been the difficulty of drugging the transcription factor. MJFF is currently funding work to develop Nurr1 agonists that can increase the activity of the target, as well as the advancement of a selective agonist into the clinic.

MJFF Supported Projects	*Active PD Companies* *($: direct or indirect funding given)*
• Acadia (2009, 2010, 2012, 2012) • Atuka (2013) • Baylor College of Medicine (2002, 2005) • Brigham & Women's Hospital (2014) • Colorado State University (2012) • Harvard Medical School (2013) • Karolinska Institutet (2006, 2007) • Lund University (2004, 2010, 2012, 2012) • Michigan State University (2016) • National and Kapodistrian University of Athens (2006, 2009) • NurrOn Pharmaceuticals (2022) • Sanofi Genzyme (2011) • University of California, SF (2019, 2022)	• Mthera Pharma (Phase 1) $ NurrOn Pharmaceuticals (Phase 1)

PARK7

(Parkinson disease protein 7/DJ1)

Novel ▸ **Emerging** ▸ Advanced   ⚡ *Pathway: oxidative stress*

Overview

Mutations in DJ-1 cause autosomal recessive PD and PD patients have higher levels of oxidized DJ-1

- *Connection to PD: expression, genetic, pathway*
- *Non-PD indications: N/A*
- *Modulation approaches: small molecule*

What's Needed?

- *Better antibodies and assays to assess the different DJ-1 species in patient samples*
- *Detailed mechanistic understanding of how alterations in oxidative stress pathways lead to PD pathogenesis*
- *Tool compounds to modulate the activity of DJ-1*
- *Robust preclinical models to test the efficacy of DJ-1 therapeutic candidates*

Target Summary

Like Parkin and PINK1, DJ-1 is associated with early-onset, recessive form of PD. DJ-1 is thought to play an important role in redox signaling pathways and regulates the transcription of anti-oxidative genes in response to stressors like aging or neuroinflammation. The crystal structure of DJ-1 has been solved, and has revealed multiple intermediate species of the protein that can now be leveraged for preclinical and clinical tool development such as antibodies and therapeutic compounds.

These studies suggest that elevating the levels of DJ-1 or augmenting its protective activity may be beneficial in PD. Alternatively, inhibiting or clearing the oxidized species of DJ-1, that is typically observed in PD patients, may be another therapeutic strategy.

MJFF Perspective

Despite the strong genetic link to PD, no major compounds that target DJ-1 have advanced to the clinic yet. Current efforts at MJFF include generation of antibodies against different DJ-1 conformations and development of tool compounds to understand the mechanisms by which DJ-1 causes neurodegeneration in PD.

MJFF Supported Projects

- Brigham & Woman's Hospital (2004)
- The Burnham Institute for Medical Research (2006)
- Cantabio Pharmaceuticals (2010, 2013, 2020)
- DiscoveRx Corp (2006)
- Doshisha University (2010, 2012)
- Labcorp (2010, 2013)
- New York University (2014)
- Paracelsus-Elena-Klinik (2010, 2012)
- The Parkinson's Institute (2004)
- PsychoGenics (2011, 2021)
- The Regents of University of Colorado (2009)
- University of Chicago (2003, 2009, 2010, 2011)
- University of Montreal (2010)
- University of Nebraska (2014)
- University of Texas (2006, 2012)
- University of Sheffield (2004)
- University of Washington (2011)
- Vrije Universiteit Amsterdam (2002)
- *Tools Development:*
  - Epitomics (2011)
  - MilliporeSigma (2009)
  - NanoTools (2015)
  - Proteos (2013, 2013, 2015)
  - Quanterix (2014, 2015, 2016)
  - SomaLogic (2015, 2016)

Active PD Companies

$ Cantabio Pharmaceuticals (Preclinical)

($: direct or indirect funding given)

*MJFF is also developing or has developed preclinical research tools for DJ-1. For more information please see our Research Tools Catalog at https://www.michaeljfox.org/research-tools-catalog

PARP1

(Poly [ADP-ribose] polymerase 1)

Novel → **Emerging** → Advanced

Pathway: epigenetics

Overview

PARP1 has been implicated in modifying alpha-synuclein to become more pathogenic
- *Connection to PD*: Pathway
- *Non-PD indications*: Autism (preclinical); Cancer (launched); Cardiovascular diseases (preclinical) Ischemia (clinical); Respiratory dysfunction (clinical)
- *Modulation approaches*: peptide, small molecule

What's Needed?

- *Replication of original high-profile results*
- *Accurate measures of CNS levels of PARylated alpha-synuclein*
- *Inhibitors with reduced cytotoxicity*

Target Summary

PARP1 has been extensively studied for its role in maintaining genome integrity as a critical factor involved in DNA damage response. Recent high-profile publications have implicated PARP1 as a target for Parkinson's disease treatment. When alpha-synuclein is "PARylated" by PARP1, it has been shown to aggregate at a faster rate than unmodified protein. Evidence from postmortem PD brains has also indicated high levels of PARylated alpha-synuclein.

MJFF Perspective

Due to the advanced stage of many PARP1 inhibitor programs in the cancer space, PARP1 inhibition is attractive as a repurposing strategy to treat Parkinson's. However, preclinical evidence needs to be replicated and strengthen prior to moving into the clinic. MJFF is funding the development of improved PARP1 inhibitors that are designed to avoid the cytotoxic effects of currently approved and in development PARP1 inhibitors

MJFF Supported Projects	Active PD Companies	*($: direct or indirect funding given)*
• Johns Hopkins University (2020) • University of Pennsylvania (2023)	• *No known companies*	

PGRN

(Progranulin)

| Novel | **Emerging** | Advanced |

Pathway: Lysosome

Overview

Neurotrophic factor that mediates various neuroprotective effects
- <u>Connection to PD</u>: genetic, pathway, expression
- <u>Non-PD indications*</u>: FTD, Corticobasal Syndrome, Primary Progressive Aphasia, Neuronal Ceroid Lipofuscinosis
- <u>Modulation approaches</u>: Gene Therapy, Small Molecule

What's Needed?

- *Mechanistic understanding of the role of PGRN in the GCase pathway and its involvement in PD*
- *Further exploring the potential of PGRN as a biomarker in PD*
- *More mature therapeutic programs targeting PGRN*

Target Summary

PGRN is expressed mainly in neurons and microglial cells and transported to the lysosome where it mediates anti-inflammation responses, neurite outgrowth, and generalized lysosomal function. It has previously been shown that a reduction in circulating Progranulin (via polymorphism or otherwise) incurs increased risk for PD development - this deficiency leads to GCase dysfunction. AZP2006 (exeprogind) interacts with Progranulin with high affinity and prevents its degradation.

MJFF Perspective

MJFF is currently funding work to assess if Progranulin modulation can rescue GCase dysfunction in a model of PD. Successful completion of this funding could provide alternative strategies to targeting the lysosomal pathway function that is a hallmark of PD.

MJFF Supported Projects	Active PD Companies ($: direct or indirect funding given)
• Alzprotect (2022) • Arkuda (2021)	• Alector (Phase 1) $ Alzprotect (Preclinical) $ Arkuda Therapeutics (Preclinical)

RABS
(RAB 29/8B/12)

(RAB29/Rab8B/Rab12, member RAS oncogene family)

Novel → **Emerging** → Advanced

Pathway: alpha-synuclein spread & endolysosome

Overview

Rab29 mutations are linked to PD and Rab29 and Rab8B protein operate in a common pathway with another PD gene- LRRK2
- *Connection to PD*: genetic, pathway, expression
- *Non-PD indications*: N/A
- *Modulation approaches*: Small molecules and gene therapy

What's Needed?

- *Mechanistic understanding of how Rab29 dysfunction causes PD*
- *Mechanistic understanding of how Rab proteins cause neurodegeneration in PD*
- *Determine feasibility of targeting rabs for PD therapeutic development*

Target Summary

Rab GTPases are master regulators of vesicular trafficking. Rab29 or Rab7L1 is one of five genes within the PARK16 locus that is linked to PD. Its recent identification as a LRRK2 substrate (along with other Rab proteins) and activator has implicated the abnormal phosphorylation of this Rab in cellular trafficking deficits. MJFF has previously supported the generation of preclinical tools and animal models to further study the contribution of dysfunctional Rab-LRRK2 signaling axis in PD pathogenesis using a variety of cellular and in vivo models.

MJFF Perspective

Current MJFF efforts are focused on novel approaches to targeting Rabs for preventing neurodegeneration in PD. MJFF is also developing immunoassays and mass-spec assays to detect phosphorylated Rabs from human biosamples to aid with patient selection for LRRK2 trials. MJFF is utilizing a similar approach to study other Rabs that have been identified as LRRK2 substrates and those that have been implicated in synuclein aggregation.

Future studies will extend these observations into relevant neuronal models of PD to confirm the mechanisms by which Rab29/Rab8B dysfunction leads to PD using a variety of approaches. These, in turn, would guide our therapeutic strategies to target this protein. It would also be of interest to determine how mutations in the PARK16 genes contribute to PD to further clarify the role of Rab29 in disease pathogenesis.

MJFF Supported Projects	Active PD Companies ($: direct or indirect funding given)

- Quanterix (2021)
- University of California, SF (2019)
- University of Dundee (2020)
- *Tools Development:*
  - Abcam (2017, 2019)
  - BioLegend (2018)
  - PEPperPRINT (2018)
  - Taconic (2018, 2019, 2019)
  - University of Dundee (2018)
  - University of Oxford (2019)
	- *No known companies*

*MJFF is also developing or has developed preclinical research tools for the Rabs. For more information please see our Research Tools Catalog at https://www.michaeljfox.org/research-tools-catalog

RIPK1

(Receptor interacting serine/threonine kinase 1)

| Novel | **Emerging** | Advanced |

Pathway: inflammation & stress response

Overview

Necroptosis pathway, involving RIPK1, is activated in PD postmortem brain tissue
- <u>Connection to PD</u>: expression, pathway
- <u>Non-PD indications*</u>: ALS (phase 1); Alzheimer's (phase 1); IBD (preclinical); Lupus (phase 1); MS (phase 1); Psoriasis (phase 1); Rheumatoid arthritis (preclinical); Unspecified inflammatory/ autoimmune diseases (preclinical)
- <u>Modulation approaches</u>:, small molecule

*Includes indications only for RIPK1 *inhibitors*

What's Needed?

- *Better compounds – current tool compounds could use improvement*
- *Preclinical validation of target*
- *More mechanistic information about which pathway RIPK1 involvement in PD – Primarily necroptosis or inflammation*

Target Summary

RIPK1 is one of the kinases that sits at the beginning of the "necroptosis" cell death pathway. Activation of the necroptosis pathway has been found in PD patient brains, along with other neurodegenerative diseases. Axonal loss is considered one of the main pathologies downstream of RIPK1 activation, although it is not completely clear if RIPK1 and necroptosis activation in dopaminergic neurons lead to the death of dopaminergic neuron axons in PD. Nec1s, a RIPK1 inhibitor, has shown positive results in vitro and in vivo models of PD.

MJFF Perspective

MJFF is currently funding work to determine if inhibition of several components of the necroptosis pathway, included RIPK1, can rescue PD pathology and behavioral outcomes in vivo pathology. This funding is also supporting genetic knockout of the downstream effector RIKP3, which at least a few companies are developing pharmacological inhibitors of.

MJFF Supported Projects	Active PD Companies ($: direct or indirect funding given)
• Universidad Mayor (2019) • Weizmann Institute of Science (2015)	• Boston Pharma/GSK (Preclinical)* • Denali Therapeutics (Phase 2)* • Sironax (Phase 1)*

*Current development not indication specific (neurodegeneration), potential for several diseases, including PD

SCD1&5

(Stearoyl-CoA desaturase)

Novel ▸ **Emerging** ▸ Advanced

Pathway: lipid metabolism

Overview

SCD inhibition rescues alpha-synuclein pathology in PD models
- *Connection to PD: expression*
- *Non-PD indications*: Cancer (preclinical); Fibrosis (clinical); Hearing Impairment (preclinical); LBD (preclinical); NASH (clinical)
- *Modulation approaches*: small molecule

*Includes indications only for SCD1 *inhibitors*

What's Needed?

- *Better pharmacodynamic and patient selection biomarkers for clinical trials*
- *Improved understanding of the SCD isoforms to target (SCD1 vs SCD5)*

Target Summary

Stearoyl-CoA Desaturases are ER enzymes that catalyze the rate-limiting step in the formation of monounsaturated fatty acids (MUFAs), including oleic acid and palmitoleic acid, which are major components of membrane phospholipids and cholesterol esters.

Lipidomic analysis on the impact of alpha-synuclein expression from yeast to human neurons have suggested that triglycerides are protective against alpha-synuclein cytotoxicity and associated ER trafficking defects by preventing the accumulation of oleic acid, and SCD inhibition has been established as a potential therapy to rescue alpha-synuclein disease-associated phenotypes.

MJFF Perspective

MJFF is funding key pre-clinical validation studies on the therapeutic efficacy of SCD1 inhibition and SCD5 knock-down as new approaches to prevent alpha-synuclein aggregation and toxicity in rodent models of PD and human neurons. The resulting data package on the best lead candidates emerging from these programs might be used to move toward with first-in-human trials in early PD subjects.

MJFF Supported Projects	*Active PD Companies* ($: direct or indirect funding given)
• Brigham & Women's Hospital (2018, 2018) • Alnylam (2021)	$ Alnylam Pharmaceuticals (Preclinical) • Janssen Pharmaceuticals (Phase 1)

STING1

(Stimulator of interferon response cGAMP interactor 1)

| Novel | **Emerging** | Advanced |

Pathway: inflammation & mitochondria

Overview

STING inhibition can rescue PINK1 and Parkin-mediated dysregulation in inflammation signaling and neurodegeneration phenotypes
- *Connection to PD*: pathway
- *Non-PD indications*: ALS (preclinical); AD (preclinical; traumatic brain injury (preclinical); AGS (clinical); Cancer (preclinical); COPD (preclinical); Huntington's (preclinical); Lupus (preclinical); Macular degeneration (preclinical); NASH (preclinical); Unspecified inflammatory/autoimmune diseases (preclinical)*
- *Modulation approaches:, small molecule*

*Includes indications only for cGAS/STING *antagonists*

What's Needed?

- *Better tool compounds to modulate STING1 and the STING/cGas pathway*
- *Further validation of the role of STING/cGas pathway in PD*
- *Preclinical tools such as KO cell lines to study the impact of STING on PD relevant phenotypes*

Target Summary

The cGAS–STING signaling axis is triggered in response to endogenous or pathogenic DNA and plays an important role in innate immune regulation. A recent study linked dysfunctional mitophagy (induced by KO of PINK1 and Parkin) to cGAS-STING activation and subsequent increases in IL-6 and IL-1β. STING activation has been shown to exacerbate neuropathology in a mouse PD model, and additional preclinical studies have shown STING as a critical driver of type-I interferon-mediated neurodegeneration. These studies suggest that decreasing the expression of STING or potentially inhibiting STING/cGAS could mitigate neuroinflammation observed in PD.

MJFF Perspective

Current MJFF efforts include generating macrophages and microglia from WT and PINK1 or Parkin KO iPSCs to investigate the PINK1/Parkin/STING axis using genetic and pharmacological approaches.
MJFF is also funding efforts to develop CNS-penetrant small molecule STING inhibitors and to conduct preclinical efficacy studies using these inhibitors in PD models.

MJFF Supported Projects	Active PD Companies (\$: direct or indirect funding given)
• AeroNeph Therapeutics (2021) • University of Oxford (2019)	\$ AeroNeph Therapeutics (Preclinical)* • IFM Due/Novartis (Preclinical)* • Ligand Pharmaceuticals (Preclinical)

*Current development not indication specific, potential for several diseases, including PD

TMEM175

(Transmembrane protein 175) 🔧

Novel **Emerging** Advanced

♻️ Ⓗ *Pathway: endolysosome & potassium channel*

Overview

Decreased function in Parkinson's presumed, affecting lysosomal pathway
- *Connection to PD: genetics, pathway*
- *Non-PD indications: N/A*
- *Modulation approaches: small molecule*

What's Needed?

- *Effect of genetic modulation of target on GCase activity evaluated in endogenous PD systems*
- *TMEM175 antibodies*

Target Summary

TMEM175 is a lysosomal potassium channel that controls lysosomal pH and clearance of autophagosomes by lysosomes. It is a genetic target linked to Parkinson's disease and variants are thought to result in loss of function of the protein and earlier disease onset. Thus, enhancing function could provide therapeutic benefit in PD.

The knowledge on structure of TMEM175, along with in vitro data on efficacy in neurons treated with synuclein PFFs and strong genetic association with the disease has made this an attractive target for PD amongst drug developers.

MJFF Perspective

MJFF is funding key functional validation studies in SHSY5Y cells and iPSC-derived dopaminergic neurons. Additionally, MJFF is supporting the development of small molecule activators and is investing in the generation of a TMEM175 antibody for quantifying and visualizing the protein.

These studies will shed light on the causative relationship between genotype and disease process and would strengthen the relevance of this target to PD. Future studies should be aimed at identifying cellular endpoints with translational value would be critical for identifying biomarkers for TMEM175 activators.

MJFF Supported Projects	Active PD Companies (\$: direct or indirect funding given)

• McGill University (2018, 2018, 2020)
• Caraway Therapeutics (2020)
• Tools Development:
   o Abcam (2020)
   o Oxford (2019)
	\$ Caraway Therapeutics/AbbVie (Preclinical)
	• Lysoway Therapeutics (Preclinical)

TRPML1/ MCOLN1

(Mucolipin TRP cation channel 1)

Novel | **Emerging** | Advanced

Pathway: lysosome

Overview

Stimulating TRPML1 can rescue lysosomal dysfunction in PD
- _Connection to PD_: pathway
- _Non-PD indications_: Lysosomal storage disorder (preclinical), Muscular dystrophy (preclinical), Niemann-Pick (preclinical)
- _Modulation approaches_: small molecule

What's Needed?

- *Understand if variants in TRPML1 are associated with PD*
- *Mechanistic studies to determine downstream players that lead to cell death/neurodegeneration in PD*
- *Identify TE and pharmacodynamic biomarkers that can be used in the clinic*

Target Summary

TRPML1 is a lysosomal calcium channel that plays an important role in regulating autophagy. The channel is mainly thought to play a role in calcium release and calcineurin-dependent TFEB nuclear translocation and lysosomal biogenesis. Loss of function mutations in TRPML1 cause the lysosomal storage disorder mucolipidosis type IV. Since mutations in lysosomal storage genes have been broadly implicated as risk factors in Parkinson's, augmenting this pathway to improve lysosomal function could be of therapeutic benefit in PD.

TRPML1 is highly druggable and cryo-EM structures of the channel can be leveraged to optimize identify and optimize available compounds. Previous studies have demonstrated that activation of TRPML1 can reset lysosomal pH to the level required for optimal hydrolase activity and this promotes clearance of accumulated materials. Similarly, overexpression of TFEB or activating TFEB using 2-hydroxypropyl-beta-cyclodextrin can induce autophagic clearance of alpha-synuclein in vitro.

MJFF Perspective

MJFF continues to support the development and advancement of CNS penetrant TRPML1 agonists as well as the development of translatable target engagement and proof-of-mechanism biomarkers.

Future efforts will focus on understanding if variants in TRPML1 are associated with PD and further preclinical efforts to understand the mechanism by which TRPML1 and TFEB impart neuroprotection in cellular and in vivo models of PD. These efforts would guide the development of preclinical tools to further understand the biology of the channel or assess target activity in patient biosamples to aid in patient enrichment and target engagement biomarkers.

MJFF Supported Projects	Active PD Companies ($: direct or indirect funding given)
• Caraway Therapeutics (2018, 2021) • Casma Therapeutics (2019) • Libra Therapeutics (2022)	• Merck/Calporta Therapeutics (Preclinical) $ Caraway Therapeutics (Preclinical) $ Libra Therapeutics (Preclinical) • Lysoway Therapeutics (Preclinical)

USP30
(Ubiquitin specific peptidase 30)

Novel ▶ **Emerging** ▶ Advanced

Pathway: cellular degradation

Overview

Target inhibits mitophagy and exhibits elevated expression seen in Parkinson's
- *Connection to PD*: pathway
- *Non-PD indications*: Fibrosis (preclinical)
- *Modulation approaches*: ASO, small molecule

What's Needed?
- *Deeper characterization of human cell lines from Parkin and PINK1 mutation carriers*
- *Expansion of preclinical tools and identification of in vitro and in vivo tool compounds*
- *Developing pharmacodynamic biomarkers to aid with clinical testing of USP30 targeted therapies*

Target Summary

USP30 is a deubiquitinating enzyme (DUB) that suppresses mitophagy by deubiquitinating mitochondrial proteins. USP30 levels are increased in human brain tissues from PD patients. Thus, decreasing expression of USP30 or inhibiting USP30 function could be beneficial in PD.

Structure of USP30 reveals a role for USP30 in cleavage of K6 ubiquitin chains and recent studies have highlighted a role for USP30 in the regulation of mitochondrial import process. Active therapeutic portfolio consists of DUB inhibitors that are extremely specific and an ASO strategy that is being explored in PD rodent models. No tool compounds are currently available but USP30 knockout mice are available and display no overt phenotype hinting at the potential safety of targeting USP30.

MJFF Perspective

Current MJFF efforts are focused on identifying the best cellular and in vivo models to test the efficacy of USP30 inhibitors. Given the role of USP30 in the mitophagy process, MJFF is funding a collaborative team to phenotype PINK1 and Parkin iPSCs under basal and stress conditions across multiple cell types. This work will aid in the identification of phenotypes crucial to the development and testing of USP30 inhibitors. MJFF is also funding labs and industry groups to test the efficacy of USP30 inhibitors in PD rodent models. In addition to USP30, MJFF is funding early target validation studies on other DUBs that have been implicated in mitophagy and synuclein aggregation. MJFF is also investing in critical preclinical tools such as antibodies against various proteins in the mitophagy pathway and supporting the characterization of animal models to test therapies targeting this pathway.

Future efforts will focus on identifying good in vivo tool compounds for the community to use and developing biomarker assays to assess target engagement and efficacy in clinical trials.

MJFF Supported Projects	Active PD Companies ($: direct or indirect funding given)
• University of Pittsburgh (2020, 2022) • University of Oxford (2020) • Mission Therapeutics (2017, 2021) • Vincere Biosciences (2019, 2022)	• Mitobridge (Preclinical)* $ Mission Therapeutics (Preclinical) • Ubiquigent (Preclinical)* $ Vincere Biosciences (Preclinical)

*Current development not indication specific, potential for several diseases, including PD

VPS13C

(Vacuolar protein sorting 13 homolog C)

Novel ▸ **Emerging** ▸ Advanced

Pathway: lysosomal & mitochondrial function

Overview

Loss-of-function mutations associated with VPS13C cause lysosomal and mitochondrial dysfunction in PD

- *Connection to PD*: genetic
- *Non-PD indications*: N/A
- *Modulation approaches*: N/A

What's Needed?

- *Mechanistic understanding of the effects of VPS13C on PD pathogenesis*
- *Better preclinical tools such as patient iPSCs and antibodies to further investigate the target in PD*
- *Diverse approaches to modulate target or the pathway on which it acts*

Target Summary

VPS13C is linked to PD through genome-wide association studies, and more recently loss-of-function mutations in the gene encoding VPS13C were shown to cause early-onset, autosomal recessive PD. Decreasing expression of VPS13C leads to mitochondrial and lipid dysfunction in a variety of cellular models.

MJFF Perspective

Current efforts include mechanistic studies to understand the impact of VPS13C on the endolysosomal system and neurodegeneration in PD and the development of novel preclinical tools to address this question.

In the future, it would be interesting to functionally characterize the protective variant of VPS13C to eventually guide drug discovery efforts against this target for PD

MJFF Supported Projects	Active PD Companies ($: direct or indirect funding given)
• Yale University (2017, 2020) • *Tools Development:* ○ University of Oxford (2019)	• *No known companies*

MJFF is also developing or has developed preclinical research tools for VPS13C. For more information please see our Research Tools Catalog at https://www.michaeljfox.org/research-tools-catalog

VPS35

(VPS35 retromer complex component)

Novel → **Emerging** → Advanced

🗑 *Pathway: autophagy/ membrane trafficking*

Overview

Mutations in VPS35 cause PD likely by altering the function of the retromer complex
- *Connection to PD*: genetic, pathway
- *Non-PD indications*: Alzheimer's (preclinical), PSP (preclinical)
- *Modulation approaches*: small molecule

What's Needed?

- *Mechanistic understanding of the effects of VPS35 on PD pathogenesis*
- *Better understanding of the clinical presentation of VPS35 mutation carriers*
- *Biomarkers that capture retromer dysfunction in VPS35 carriers and iPD subjects*
- *Tool compounds to modulate target/pathway*

Target Summary

VPS35 is a cause of late-onset , autosomal dominant Parkinson's disease and a single mutation (D620N) has been identified as the major cause for disease manifestation. VPS35 is a core component of the retromer that plays an important role in membrane trafficking. More recently, the D620N mutation in VPS35 was shown to enhance LRRK2 kinase activity in non-neuronal models. Taken together, these studies hint at the involvement of the retromer in mediating neurodegeneration due to defective trafficking and recycling of key endosomal cargoes.

MJFF Perspective

Current MJFF efforts include understanding the downstream pathogenic processes impacted by VPS35 PD mutations.

Future efforts will focus on mechanistic studies in different cell types using iPSC and rodent models to better map out the pathogenic processes that play a role in VPS35 PD. Future efforts will also be directed at better understanding retromer dysfunction in patient populations with VPS35 mutations and expanding these assays to identify iPD populations who present with dysfunction in retromer/endolysosomal system for expanding the pool of patients who would benefit from retromer-targeted therapies.

MJFF Supported Projects	Active PD Companies ($: direct or indirect funding given)

• Columbia University (2021)
• EPFL (2012)
• Garvan Institute of Medical Research (2020)
• Van Andel Research Institute (2016, 2018)
• Temple University (2016)
• University of Dundee (2018, 2018, 2020, 2020)
• University of Minnesota (2020)
• University of Montreal (2020)
• *Tools Development:*
    ○ Ozgene Pty Ltd (2011)
    ○ University of Oxford (2019)
	• Retromer Therapeutics (Preclinical)

*MJFF is also developing or has developed preclinical research tools for VPS35. For more information please see our *Research Tools Catalog* at https://www.michaeljfox.org/research-tools-catalog*

ADVANCED TARGET PROFILES

A target that has strong links to Parkinson's disease through genetics/expression/known cellular pathways AND target modulation data in endogenous PD models AND sufficient interest from stakeholders at academia/industry/CROs

GBA *(β-Glucocerebrosidase)* 💉

Novel > Emerging > **Advanced**

🔬 *Pathway: cellular degradation*

Overview

Mutations in GBA1 linked to PD cause accumulation of key lipids that are important for lysosomal health

- *Connection to PD*: genetic
- *Non-PD indications*: ALS (clinical); Alzheimer's (clinical); FTD (preclinical); Fabry's (clinical); Gaucher's (launched); LBD (preclinical); Lysosomal storage disorder (clinical); Niemann-Pick (launched)
- *Modulation approaches*: antibody, cell therapy, gene therapy, small molecule

What's Needed?

- *Better understanding of how PD-linked mutations cause changes in GCase function*
- *Preclinical tools to assess GCase activity and levels*
- *Tool compounds to modulate GCase activity*
- *Characterization of various PD-GBA models to assess pharmacodynamic changes and efficacy of GBA-targeted therapies*
- *Understanding the lipid changes (global and sphingolipid) in GBA-linked PD*
- *Biomarkers to assess TE in the CNS and for patient enrichment*

Target Summary

Mutations in the glucocerebrosidase (GBA1) gene are common risk factors for Parkinson's disease. GBA1 mutations affect GCase structure and reduce its enzymatic function, leading to the accumulation of glycosphingolipids and impaired lipid homeostasis. The mechanism by which GBA1 mutations are linked to PD is still poorly understood. However studies in preclinical models have suggested three main hypothesis: 1) A loss of function hypothesis where the accumulation of lipids resulting from the reduced GCase activity directly affect trafficking, processing, and clearance of aSyn, resulting in its accumulation and aggregation; 2) a gain-of-function hypothesis suggesting that misfolded GCase directly interacts with aSyn, leading to its accumulation; and 3) a bidirectional loop in which GCase deficiency facilitates aSyn oligomerization which, in turn, impairs GCase activity. Additionally, it has been reported that GBA1 mutations can result in mitochondrial dysfunction, ER stress, and neuroinflammation. A major controversy in the field regards the fact that, despite the unequivocal correlation between the presence of GBA1 mutations and the risk of developing PD, only a minority of carriers with GBA1 mutations convert to PD in their lifetime.

MJFF Perspective

GCase is a target genetically linked to Parkinson's disease but has a relatively low penetrance. MJFF is interested in identifying genetic and non-genetic modifiers of GCase and associated lysosomal pathways with the aim of further understanding the molecular mechanism of disease in GBA-PD, nominate additional targets with therapeutic potential for GBA-PD, and refine patient enrichment strategies for identifying at-risk populations.

In order to enrich the therapeutic pipeline for GBA-PD, MJFF is currently supporting structural biology studies for GCase (wildtype, mutants, and in association with interacting partners) to develop better therapeutic approaches with the aim of restoring GCase function. These include small molecules, biologics, and gene therapy.

GBA

(β-Glucocerebrosidase) 💉

Novel	Emerging	**Advanced**

Pathway: cellular degradation

Overview

Mutations in GBA1 linked to PD cause accumulation of key lipids that are important for lysosomal health

- *Connection to PD*: genetic
- *Non-PD indications*: ALS (clinical); Alzheimer's (clinical); FTD (preclinical); Fabry's (clinical); Gaucher's (launched); LBD (preclinical); Lysosomal storage disorder (clinical); Niemann-Pick (launched)
- *Modulation approaches*: antibody, cell therapy, gene therapy, small molecule

What's Needed?

- *Better understanding of how PD-linked mutations cause changes in GCase function*
- *Preclinical tool to assess GCase activity and levels*
- *Tool compounds to modulate GCase activity*
- *Characterization of various PD-GBA models to assess pharmacodynamic changes and efficacy of GBA-targeted therapies*
- *Understanding the lipid changes (global and sphingolipid) in GBA-linked PD*
- *Biomarkers to assess TE in the CNS and for patient enrichment*

---

*Active PD Companies**

($: direct or indirect funding given)

- Alector (Phase 1)
- $ Arkuda Therapeutics (Preclinical)
- Aspen Neuroscience (Preclinical)
- Bioasis (Preclinical)
- BIAL (Phase 1)
- $ Caraway Therapeutics (Preclinical)
- Chamishi Therapeutics (Preclinical)
- Coave Therapeutics (Preclinical)
- Eli Lilly/Prevail (Phase 1/2)
- $ Gain Therapeutics (Phase 1)

- Q-State Bio (Preclinical)
- Neurocrine (Preclinical)
- Regenxbio (Phase 1/2)
- Sharp Therapeutics (Preclinical)
- $ Sinfonia Biotherapeutics (Preclinical)
- $ Vanqua Bio (Preclinical)
- Voyager Therapeutics (Preclinical)

*Companies codeveloping therapies are listed separately

MJFF is also developing or has developed preclinical research tools for GBA1. For more information please see our *Research Tools Catalog* at *https://www.michaeljfox.org/research-tools-catalog*

LRRK2   *(Leucine Rich Repeat Kinase 2)*

Novel ⟩ Emerging ⟩ **Advanced**   ♻ *Pathway: endolysosome*

Overview

Hyperactivation of LRRK2 caused by mutations in the gene play a key role in PD
- <u>Connection to PD</u>: genetic
- <u>Non-PD indications</u>: ALS (preclinical); Cancer (clinical); Crohn's (preclinical); Glaucoma (clinical); Hypertension (preclinical)
- <u>Modulation approaches</u>: ASOs, RNA-editing, small molecule

What's Needed?

- *Structures of active and inactive LRRK2*
- *Mechanisms downstream of Rabs that contribute to neurodegeneration*
- *Other approaches to targeting LRRK2*
- *Understanding the role of risk-variants in PD*
- *Patient enrichment biomarkers for LRRK2 clinical trials*
- *Target engagement biomarkers for the CNS*

Target Summary

Mutations in LRRK2 are the most common genetic cause of both familial and sporadic PD. Clinically, LRRK2-PD is believed to be indistinguishable from iPD and more recently, studies have indicated a role for elevated expression and activity of LRRK2 in iPD. Based on preclinical evidence demonstrating neuroprotection upon inhibition of LRRK2 activity or reduction of LRRK2 expression, LRRK2 kinase inhibitors and ASOs have been developed and advanced to the clinic.

As we await outcomes of ongoing clinical trials, MJFF continues to support the identification and validation of patient enrichment biomarkers and CNS engagement biomarkers. Additionally, we are continuing to collaborate with industry and academic groups to test novel approaches to targeting LRRK2 and identifying other drug targets that can modify LRRK2 expression and/or activity.

MJFF Perspective

Given its strong genetic link to PD and compelling therapeutic rationale, MJFF has been a consistent supporter and funder of studies to facilitate translation of LRRK2 research into treatments for people with PD.

MJFF has used a highly collaborative model to support work around key challenges and barriers to progress. The outcomes of these efforts are evident in the growing number of LRRK2 therapeutic programs moving closer to or currently in clinical trials.

*Active PD Companies**   *($: direct or indirect funding given)*

- 1st Biotherapeutics (Preclinical)
- $ Ambagon Therapeutics (Preclinical)
- Arvinas (Preclinical)
- $ Biogen (Phase 1 & Phase 3)
- Cerevel (Preclinical)
- $ Denali Therapeutics (Phase 3)
- Halia Therapeutics (Preclinical)
- $ Ionis Pharmaceuticals (Phase 1)
- $ Merck (Preclinical)
- Neuron23 (Phase 1)
- Oncodesign Biotechnology (Preclinical)
- Servier (Preclinical)
- Shape Therapeutics (Preclinical)

*Companies codeveloping therapies are listed separately

MJFF is also developing or has developed preclinical research tools for LRRK2. For more information please see our *Research Tools Catalog at https://www.michaeljfox.org/research-tools-catalog*

NLRP3

(NLR family pyrin domain containing 3)

Novel	Emerging	**Advanced**

Pathway: inflammation

Overview

Target activates inflammation with increased expression seen in PD

- *Connection to PD: expression*
- *Non-PD indications: Alzheimer's (preclinical); ALS (preclinical); Arthritis (preclinical); Cancer (clinical); Cytokine release syndrome (clinical); Dermatology (clinical); Heart failure (clinical); IBD (preclinical); Pain (clinical); MS (preclinical)*
- *Modulation approaches: antibody, small molecule*

What's Needed?

- *Assays to assess NLRP3 activation in patients*
- *More knowledge of time-course of NRLP3 activation during disease progression*
- *More specific, centrally-penetrant inhibitors*

Target Summary

NLRP3 is part of an inflammasome complex, which when activated, triggers many inflammatory pathways that are implicated in Parkinson's disease pathology. The NLRP3 inflammasome has been explored as a therapeutic target in multiple peripheral inflammatory conditions, and is implicated in neurodegenerative diseases, including Parkinson's. Increased NLRP3 expression has been reported in human PD brains. Genetic and pharmacological inhibition of NLRP3 activation has shown positive results in multiple preclinical models of PD. The first phase 1 clinical trial of a centrally-penetrant NLRP3 inhibitor showed no safety concerns preventing further development

MJFF Perspective

A major gap in our knowledge around NLRP3 is the development of reliable tools that can be used to stratify patients by levels of NLRP3 activation, as well as directly assess central as well as peripheral target engagement of the NLRP3 inflammasome. To this end, MJFF recently funded work on the development of an assay for determining NLRP3 activation in blood, which provided mixed results. MJFF is also currently funding work on an NLRP3 PET-tracer.

Clinical validation of NLRP3 in PD is the next step for this target. There are also questions around if central inhibition of NLRP3 is needed for efficacy, or peripheral inhibition is sufficient. MJFF may fund work to address this question, which is vital since there are multiple companies with peripherally-restricted NLRP3 inhibitors in development. MJFF will continue to support biomarker and biology work around NRLP3 where appropriate to support clinical development.

MJFF is also developing or has developed preclinical research tools for NLRP3. For more information please see our *Research Tools Catalog at https://www.michaeljfox.org/research-tools-catalog*

NLRP3

(NLR family pyrin domain containing 3)

Novel ＞ Emerging ＞ **Advanced**

Pathway: inflammation

Overview

Target activates inflammation with increased expression seen in PD

- *Connection to PD: expression*
- *Non-PD indications: Alzheimer's (preclinical); ALS (preclinical); Arthritis (preclinical); Cancer (clinical); Cytokine release syndrome (clinical); Dermatology (clinical); Heart failure (clinical); IBD (preclinical); Pain (clinical); MS (preclinical)*
- *Modulation approaches: antibody, small molecule*

What's Needed?

- *Assays to assess NLRP3 activation in patients*
- *More knowledge of time-course of NRLP3 activation during disease progression*
- *More specific, centrally-penetrant inhibitors*

MJFF Supported Projects

- AC Immune (2021)
- Dartmouth College (2015, 2017, 2021)
- EpicentRx (2021)
- Inflazome (2019)
- Institut du Cerveau (ICM) (2016, 2018)
- Olatec (2022)
- Quanterix (2021)
- Roche (2021)
- University of Queensland (2014, 2016, 2016)

Active PD Companies   *($: direct or indirect funding given)*

- $ AC Immune (Preclinical)*
- • Adiso Therapeutics (Preclinical)*
- • Asha Therapeutics (Preclinical)*
- • BioAge Labs (Preclinical)*
- $ EpicentRx (Preclinical)*
- • Halia Therapeutics (Preclinical)*
- • IMMvention Therapeutics (Preclinical)*
- • Mabylon (Preclinical)*
- • Neumora Therapeutics (Preclinical)*
- • NodThera (Preclinical & Phase 1)*
- • Novartis/IFM Tre (Preclinical)*
- $ Olatec (Preclinical)*
- • Roche/Inflazome (Preclinical & Phase 1)
- • Ventus Therapeutics (Phase 1)*
- • Ventyx Biosciences (Phase 1)*
- $ Zyversa Therapeutics (Preclinical)*

*Current development not indication specific, potential for several diseases, including PD

PINK1

(Serine/Threonine-Protein Kinase PINK1)

Novel > Emerging > **Advanced**

Pathway: cellular degradation

Overview

Target plays a role in mitochondrial QC pathways and loss-of-function mutations in PINK1 cause early-onset PD

- *Connection to PD*: genetic
- *Non-PD indications*: Alzheimer's (preclinical); Huntington's (preclinical)
- *Modulation approaches*: gene therapy, small molecule

What's Needed?

- *Knowledge of mechanisms by which PINK1 causes neurodegeneration (Parkin dependent and independent mechanisms)*
- *Expression and activity pattern of PINK1 in rodents and humans*
- *Tools and assays to measure activation*
- *Animal models to assess pharmacodynamic changes and efficacy*
- *In vitro and in-vivo tool compounds to further validate mechanisms*
- *Structure of human protein*

Target Summary

PINK1 is an autosomal recessive gene that causes early-onset Parkinson's disease. Genetic and biochemical studies have revealed that PINK1 is upstream of Parkin and plays a role in the clearance of damaged mitochondria through a process termed "mitophagy". Like Parkin, PINK1 activation suppresses miTAP and the STING/cGas pathway. Loss of PINK1 can lead to cell loss and neurodegeneration through disruption of mitophagy, miTAP and activation of the STING/cGAS pathway. This detailed understanding has led to the development of PINK1 activators for the treatment of Parkinson's disease.

Current efforts at MJFF are focused on establishing PINK1 patient cohorts to understand phenotypes and clinical progression in these individuals. Current efforts are also focused on establishing animal models to test PINK1 therapies for assessing efficacy and establishing translational biomarkers that could facilitate clinical testing of PINK1 activators.

MJFF Perspective

PINK1 is a target genetically linked to Parkinson's disease. Given our knowledge about the mitochondrial mechanisms in PD and the role of PINK1 in regulating mitophagy, there is a strong rationale to develop drugs targeting the PINK1 pathway.

MJFF continues to generate preclinical and clinical resources to identify new druggable targets within the PINK1/Parkin pathway, enables the identification and validation of PINK1 pathway biomarkers and facilitates collaborative studies to address the translational gaps for this target

Active PD Companies   *($: direct or indirect funding given)*

- Amathus Therapeutics (Preclinical)
- $ Mitokinin (Preclinical)

MJFF is also developing or has developed preclinical research tools for PINK1. For more information please see our *Research Tools Catalog at https://www.michaeljfox.org/research-tools-catalog*

PRKN

(parkin RBR E3 ubiquitin protein ligase) 💉

Novel	Emerging	**Advanced**

🔬 *Pathway: cellular degradation*

Overview

Target plays a role in mitochondrial QC pathways and loss-of-function mutations in Parkin are the most common cause of early-onset PD

- *Connection to PD: genetic*
- *Non-PD indications: Alzheimer's (preclinical); Cancer (preclinical)*
- *Modulation approaches: gene therapy, small molecule*

What's Needed?

- *Expression and activity pattern of Parkin in rodents and humans*
- *Tools and assays to measure Parkin pathway activation*
- *Understanding mechanisms linking Parkin-PD to iPD*
- *Animal models to assess pharmacodynamic changes and efficacy*
- *In vitro and in-vivo tool compounds to further validate mechanisms*

Target Summary

Parkin is an autosomal recessive gene that causes early-onset Parkinson's disease.
Genetic and biochemical studies have revealed that Parkin is essential for the clearance of damaged mitochondria through a process termed "mitophagy". More recently, Parkin has also been implicated in regulating immune function through suppression of miTAP and the STING/cGas pathway. Loss of Parkin can lead to cell loss and neurodegeneration through disruption of mitophagy, miTAP and activation of the STING/cGAS pathway. This detailed understanding has led to the development of Parkin activators for the treatment of Parkinson's disease.

Current efforts at MJFF are focused on establishing Parkin patient cohorts to understand phenotypes and clinical progression in these individuals. Current efforts are also focused on establishing animal models to test Parkin therapies for assessing efficacy and establishing translational biomarkers that could facilitate clinical testing of Parkin activators.

MJFF Perspective

Parkin is a target genetically linked to Parkinson's disease. Knowledge of the structure of the protein and the detailed biological mechanisms contributing to neurodegeneration have provided a strong rationale for developing Parkin-targeted therapies.

As gene-therapy approaches and small molecule activators continue to be generated and tested, MJFF continues to provide preclinical and clinical resources to the community to enable biomarker development and facilitates collaborative studies to address the translational gaps for this target

Active PD Companies	*($: direct or indirect funding given)*
$ Cellivery (Preclinical)	$ Progenra (Preclinical)
$ Eisai (Preclinical)	$ Vincere Biosciences (Preclinical)
$ NysnoBio (Preclinical)	

MJFF is also developing or has developed preclinical research tools for PRKN. For more information please see our *Research Tools Catalog at https://www.michaeljfox.org/research-tools-catalog*

SNCA

(Alpha-synculein)

Novel ▸ Emerging ▸ **Advanced**

Pathway: Multiple (protein aggregation)

Overview

Genetic and pathological evidence link SNCA to PD

- <u>Connection to PD</u>: expression, genetic
- <u>Non-PD indications</u>: DLB (preclinical); MSA (clinical)
- <u>Modulation approaches</u>: antibody, gene therapy, small molecule, RNA-editing vaccine

What's Needed?

- *Quantitative assays to measure pathological/aggregated forms of alpha-synuclein*
- *Patient-enrichment strategies for alpha-synuclein targeted therapies*
- *Better antibodies to detect pathological species of synuclein*
- *Better preclinical models of alpha-synuclein pathology*
- *Better understanding of the relevance of synuclein propagation in PD (peripheral and central)*

Target Summary

Classically considered a natively unfolded protein, aSyn has the ability to undergo aggregation and form insoluble amyloid fibrils that are a major component of the Lewy bodies and Lewy neurites found in PD, DLB, and MSA.

Multiple mechanisms have been proposed linking aSyn aggregation and accumulation with PD pathogenesis including mitochondrial dysfunction, compromised autophagy, early synaptic alterations, inflammation and immune response, etc. Most are compelling and consistent with select aspects of PD pathology recapitulated in rodent models of PD and supported by studies of postmortem human PD brain. In addition, the clinical observations that the presence of Lewy pathologies in the brain generally follow a defined pattern that correlates with the stage of disease development, led to the concept that aSyn itself is spreading from cell to cell in a prion-like manner driving the disease progression.

This biological understanding of aSyn pathobiology has led to the development of multiple therapeutic approaches aiming a reducing the dosage, aggregation, and spreading of aSyn including targeting SNCA expression through gene repression and translation blockers, aSyn aggregation through aggregation inhibitors/modifiers and monomer stabilizers, active and passive immunotherapy approaches to halt aSyn spreading, and inducers of aSyn degradation through autophagy and/or the proteosome.

MJFF Perspective

See Next Page

MJFF is also developing or has developed preclinical research tools for SNCA. For more information please see our *Research Tools Catalog at* https://www.michaeljfox.org/research-tools-catalog

SNCA

(Alpha-synculein)

Novel 〉 Emerging 〉 **Advanced**

Pathway: Multiple (protein aggregation)

Overview

Genetic and pathological evidence link SNCA to PD

- *Connection to PD*: expression, genetic
- *Non-PD indications*: DLB (preclinical); MSA (clinical)
- *Modulation approaches*: antibody, gene therapy, small molecule, RNA-editing vaccine

What's Needed?

- *Quantitative assays to measure pathological/aggregated forms of alpha-synuclein*
- *Patient-enrichment strategies for alpha-synuclein targeted therapies*
- *Better antibodies to detect pathological species of synuclein*
- *Better preclinical models of alpha-synuclein pathology*
- *Better understanding of the relevance of synuclein propagation in PD (peripheral and central)*

MJFF Perspective

Although whether aSyn causes PD is not known, the generally quite defined pattern of aSyn pathology in PD, the disease phenotypes observed in cellular and animal models of aSyn overexpression and fibril formation, in combination with the human genetic data and postmortem studies suggest that this aberrant deposition of aSyn is a major driving force in PD pathogenesis.

Despite the large body of evidence linking aSyn and PD in preclinical models, testing the various therapeutic hypothesis around aSyn pathobiology in human clinical trials has been challenging. MJFF efforts are multi-layered and aimed at facilitating the translation of aSyn therapeutics into the clinic. This includes identifying and characterizing modifiers of aSyn pathobiology, assessing the safety of lowering aSyn in neurons, investigating the role of aSyn strains and PTMs, understanding aSyn's role in the periphery and immune system, correlating aSyn pathobiology and disease progression, investigating the mechanisms of pathological aSyn spreading and clearance, and the connection of aSyn and lipid homeostasis. Funding research in these areas is accompanied by a robust pipeline of validated preclinical tools to de-risk and standardize research across the aSyn field.

Important for designing clinical trials that allow testing the therapeutic hypothesis, MJFF has put a strong effort in developing biomarkers of disease progression, diagnosis, and subtyping, through a robust project pipeline ranging from development and validation of aSyn assays and imaging approaches to measure pathological aSyn in the brain, biofluids, and peripheral tissues, to the clinical validation and qualification of biomarkers in MJFF-sponsored cohorts. In this regard we're currently exploring the power of seed amplification assays to detect minute amounts of aSyn pathological species in human samples while supporting the development of quantitative SAAs and refining methods for quantifying total and various disease-relevant species of aSyn (e.g., pS129-aSyn).

SNCA

(Alpha-synuclein)

Novel ⟩ Emerging ⟩ **Advanced**

Pathway: Multiple (protein aggregation)

Overview

Genetic and pathological evidence link SNCA to PD
- *Connection to PD*: expression, genetic
- *Non-PD indications*: DLB (preclinical); MSA (clinical)
- *Modulation approaches*: antibody, gene therapy, small molecule, RNA-editing vaccine

What's Needed?

- *Quantitative assays to measure pathological/aggregated forms of alpha-synuclein*
- *Patient-enrichment strategies for alpha-synuclein targeted therapies*
- *Better antibodies to detect pathological species of synuclein*
- *Better preclinical models of alpha-synuclein pathology*
- *Better understanding of the relevance of synuclein propagation in PD (peripheral and central)*

Active PD Companies*

($: direct or indirect funding given)

- ABL Bio (Preclinical)
- $ AC Immune (Preclinical, Ph1, Ph2)
- $ Alterity Therapeutics (Phase 1)
- AltPep (Preclinical)
- $ Amydis (Preclinical)
- $ Aprinoia Tx (Preclinical)
- Arvinas (Preclinical)
- AstraZeneca (Phase 2)
- $ Axial Therapeutics (Preclinical)
- BioArctic (Phase 1)
- $ Biogen (Preclinical)
- Chimerna Therapeutics (Preclinical)
- Eisai (Preclinical)
- Enterin (Phase 2)
- ICBI (Preclinical)

- $ GISMO Therapeutics (Preclinical)
- $ Lundbeck (Phase 1)
- $ Merck (Phase 1)
- $ MODAG (Phase 1)
- $ ND Biosciences (Preclinical)
- Neuramedy (Preclinical)
- $ Neuropore (Phase 2)
- $ Nitrase Therapeutics (Preclinical)
- Novartis (Phase 2)
- Prazer Therapeutics (Preclinical)
- $ Priavoid (Preclinical)
- ProMIS (Preclinical)
- Prothena (Phase 2)
- Roche (Phase 2)
- Sanofi (Phase 1)

- SciNeuro (Preclinical)
- $ Seelos Therapeutics (Preclinical)
- $ Syngle Therapeutics (Preclinical)
- Takeda (Phase 2)
- Treventis (Preclinical)
- UCB (Phase 1 & Phase 2)
- UniQure (Preclinical)
- $ Vaxxinity (Phase 1)
- VectorY Therapeutics (Preclinical)
- Wren Therapeutics (Preclinical)

*Companies codeveloping therapies are listed separately

TLR2

(Toll like receptor 2)

| Novel | **Emerging** | Advanced |

Pathway: inflammation

Overview

TLR2 modulates inflammation and is upregulated in samples from human PD patients

- *Connection to PD*: expression, pathway
- *Non-PD indications**: ALS (preclinical); Asthma (preclinical); Atherosclerosis (phase 2); Hearing loss (preclinical); Infection (preclinical); NASH/Fibrosis (preclinical); Sepsis (preclinical)
- *Modulation approaches*: peptide, small molecule

*Includes indications only for TLR2 *antagonists*

What's Needed?

- *Further mechanistic information on TLR2 role in autophagy in neurons*
- *Clinical validation of TLR2 inhibition in PD*

Target Summary

TLR2 is a toll-like receptor that is responsible for the detection of inflammatory stimuli. TLR2 levels are increased in the brains of PD patients and alpha-synuclein transgenic mice. Genetic ablation of TLR2 reduces inflammation in mouse models of PD, as does treatment with TLR2-inhibiting antibodies.

MJFF Perspective

With MJFF funding, Neuropore therapeutics developed brain-penetrant small molecule TLR2 inhibitors that reduced alpha-synuclein induced inflammation in vitro and in vivo. The company also developed an ex vivo assay to assess target engagement, which could potentially be used in clinical trials.

Future efforts should focus on further assessments of TLR2's safety as a target, and the safety of TLR2 inhibitor treatment in higher species. TLR2 target engagement assays should be finalized prior to entry into the clinic.

MJFF Supported Projects	Active PD Companies (\$: direct or indirect funding given)
• Neuropore Therapies (2017, 2018, 2022) • Opsona Therapeutics (2009)	• Aptamer (Preclinical) \$ Neuramedy (Preclinical) \$ Neuropore (Preclinical)