Medical Need in Neuroscience is Still High and is Growing with the Aging Population

- Alzheimer’s Disease
- Parkinson’s Disease
- ALS
- Schizophrenia
- MENTAL HEALTH
- Sleep Disorders
- REM vs. Awake
- Pain
Delivering Innovation in Neuroscience

**Past**

**Product-focused**

- Psychiatry
  - Empirically-derived / iterative
  - Symptoms targeted broadly

- Neurology
  - Pathophysiology not known
  - Symptomatic treatments
  - Focus on key DAs: Alzheimer’s/Parkinson’s, Pain
  - Target and pathway driven
  - Disease modifying and symptomatic treatments
  - Hypothesis-focused testing
  - Biomarkers
  - Translational models
  - Testing molecules in the clinic (i.e. multiple shots on goal)
  - Molecules used to validate animal models
  - Candidates competed internally for development

**Future**

**Customer-centric**

- Mono-therapy and augmentation strategies
- Novel mechanisms
- Defined symptoms / outcomes
- Focus on key DAs: Alzheimer’s/Parkinson’s, Pain
- Target and pathway driven
- Disease modifying and symptomatic treatments
- Partnerships for technology, capacity, or expertise (Industry / Academics / Government)
- Hypothesis-focused testing
- Biomarkers
- Translational models
- Multiple Avenues for compound Development

**Transform**

- Refocus Psychiatry
- Broadening focus on Neurological Diseases
- Novel Technologies to Increase Productivity
- Partnerships Leverage External Environment
- Creative Early Development Approaches

**How are we doing?**

**What have “we” learned?**
Using Human Biology to Shape Therapeutic Approaches and Select the Best Drug Targets

What's in our toolbox?

Genetics

Pathophysiology

Pharmacology

Understanding of human disease

New ideas for drug targets

Modify/prevent disease

Restore function
Using Human Biology to Shape Therapeutic Approaches and Select the Best Drug Targets

What's in our toolbox?

- Genetics
- Pathophysiology
- Pharmacology

Understanding of human disease

New ideas for drug targets

1. Modify/prevent disease

Example: target pathophysiology
- Tau “Tangle”
- Amyloid “Plaque”

2. Restore function

Example: Restoring acetylcholine signaling
- Normal Brain
- Alzheimer’s Brain

Cholinergic Neurons
- Vehicle
- Compound
- Receptor PAMs Nicotinic Muscarinic
The BACE Inhibitor (MK-8931) Will Test the Leading Alzheimer’s Hypothesis

1906

Plaque and Tangle Pathology Described by Alois Alzheimer

1984

Isolate Aβ peptides from Alzheimer plaques

1988

Amyloid Precursor Protein (APP) Cloned

1990s

Familial AD genes discovered
“Amnloid Hypothesis”

1999

BACE Cloned

1999

BACE Inhibitor Programs Initiated

2011

MK-8931 Lowers Aβ peptides in Human CSF

2012

EPOCH Pivotal Trial Initiated

Genetics Support for BACE inhibition

Protective APP Mutation at BACE Cleavage Site
Clinical Doses of MK-8931 (Verubestat) Have a Major Impact on CSF / Brain Aβ

Merck is proud to announce the approved generic name for its investigational BACE inhibitor MK-8931...

**Verubecestat**

Clinical trials for this investigational drug are underway.

**MK-8931 AD Subjects**

- Placebo
- MK-8931 12 mg
- MK-8931 40 mg
- MK-8931 60 mg

<table>
<thead>
<tr>
<th>Time (Days)</th>
<th>Baseline</th>
<th>0</th>
<th>4</th>
<th>8</th>
<th>12</th>
<th>16</th>
<th>20</th>
<th>24</th>
<th>28</th>
<th>32</th>
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<tbody>
<tr>
<td>CSF Aβ40 % of Baseline</td>
<td>0</td>
<td>40</td>
<td>80</td>
<td>100</td>
<td>120</td>
<td>160</td>
<td></td>
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</table>

Day -1
0 hours
Day 7 Dose
Day 7 Dose
Co-primary endpoints: change from baseline (model-based mean ± SE)

**ADAS-Cog11**
Treatment (observed baseline mean ± SD)
- 12 mg (21.3 ± 7.5)
- 40 mg (21.4 ± 7.6)
- Placebo (21.7 ± 7.6)

**ADCS-ADL**
Treatment (observed baseline mean ± SD)
- 12 mg (63.1 ± 9.4)
- 40 mg (62.9 ± 9.9)
- Placebo (62.1 ± 10.5)

**Count Table**

<table>
<thead>
<tr>
<th></th>
<th>12 mg</th>
<th>40 mg</th>
<th>Placebo</th>
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<tbody>
<tr>
<td>12 mg</td>
<td>629</td>
<td>621</td>
<td>639</td>
</tr>
<tr>
<td>40 mg</td>
<td>619</td>
<td>616</td>
<td>628</td>
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<tr>
<td>Placebo</td>
<td>607</td>
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<tr>
<td></td>
<td>448</td>
<td>432</td>
<td>463</td>
</tr>
</tbody>
</table>

**Week 78 Difference**

- 12 mg vs. placebo  \( p = 0.63 \)
- 40 mg vs. placebo  \( p = 0.46 \)

Jun 2018 - Early termination (futility) - Lanabecestat (BACE inhibitor; Prodromal/Mild AD; AMARANTH and DAYBREAK)
May 2018 – Liver tox (safety) - Atabecestat (BACE inhibitor; Subjects at risk of AD; EARLY)
Apr 2018 – Did not meet primary endpoint – Azeliragon (RAGE inhibitor; Mild AD; STEAFAST)
Feb 2018 - Early termination (futility) - Verubecestat (BACE inhibitor; Prodromal AD; APECS)
Jan 2018 - Early termination (futility) – Pioglitazone (PPARγ agonist; onset to MCI; TOMORROW)
Feb 2017 - Early termination (futility) - Verubecestat (BACE inhibitor; Mild to Moderate AD; EPOCH)
Jan 2017 - Terminated early - Solanezumab (Abeta antibody; prodromal AD; EXP-PRO)
Nov 2016 - Failed – Solanezumab (Abeta antibody; mild AD; EXPIII)

Alzheimer's Drug Trials Keep Failing -- It May Be Because We Don't Understand the Disease
The theory of what causes the disease and how it develops may be wrong.
Using Human Genetics to Shape Therapeutic Approaches?

Understanding of human disease

Alzheimer’s Disease
APP (amyloid precursor protein)
PSEN1/2 (presenilin 1 & 2)
APOE4 (apolipoprotein E)
APP BACE cleavage site
TREM2 (Triggering receptor expressed on myeloid cells 2)

Important to also understand the biology of the gene linked to the disease
Using Human Genetics to Shape Therapeutic Approaches and Select the Best Drug Targets

Understanding of human disease

How are we doing? What have “we” learned?

Genetics

Alzheimer’s Disease
- APP (amyloid precursor protein)
- PSEN1/2 (presenilin 1 & 2)
- APOE4 (apolipoprotein E)
- APP BACE cleavage site
- TREM2 (Triggering receptor expressed on myeloid cells 2)

Parkinson’s Disease
- SNCA (alpha synuclein)
- GBA (glucocerebrosidase)
- LRRK2 (Leucine Rich Repeat Kinase 2)

Amyotrophic Lateral Sclerosis (ALS)
- SOD1 (superoxide dismutase 1)
- TDP43 Protein (TAR DNA-binding protein 43)
- C9orf72 (chromosome 9 open reading frame 72)
Leucine Rich Repeat Kinase 2 (LRRK2)
• LRRK2 (PARK8) mutations are the most common autosomal dominant cause of PD
  – 5–13% of familial PD, 1–3% of idiopathic PD
  – Disease penetrance: 59 yrs (28%), 69 yrs (51%), and 79 yrs (74%)
• LRRK2 driven PD is clinically and pathologically indistinguishable from idiopathic PD
• Mutations in or near the “enzymatic core” – pathogenic gain-of-function

LRRK2 Structure

Inhibition of LRRK2 kinase activity represents a tractable and novel therapeutic target to modify the progression of Parkinson’s disease
Using Human Biology to Shape Therapeutic Approaches and Select the Best Drug Targets

LRKK2

α-synuclein pathology
lysosomal function

PD Risk Gene

Human Genetics

Biology

Enzymes

Druggability
Discovery of a novel, potent and selective LRRK2 kinase inhibitor series (Indazole Core)

Exemplar HTS Hit
LRRK2 IC\textsubscript{50}: 27 nM
Cell LRRK2 pS935 IC\textsubscript{50}: N.D.

Compounds

Compound A
LRRK2 IC\textsubscript{50}: 2 nM
Cell LRRK2 pS935 IC\textsubscript{50}: 22–64 nM

MLi-2
LRRK2 IC\textsubscript{50}: 1 nM
Cell LRRK2 pS935 IC\textsubscript{50}: 4 nM

Optimization of LRRK2 kinase inhibitor hit identifies MLi-2 as a highly potent and selective inhibitor of LRRK2
Pharmacological characterization of the novel, potent and selective LRRK2 kinase inhibitor MLi-2

MLi-2
- Potent inhibitor of LRRK2 kinase activity in vivo
- In vivo brain (mouse) pSer935 IC$_{50}$ = 0.8 nM
- Well tolerated in mice after chronic dosing with >95% CNS target engagement

MLi-2 is an excellent molecule to probe the biology of LRRK2 kinase inhibition in vivo
Chronic in-diet dosing of Mli-2 does not attenuate the behavioral phenotype of the MitoPark mouse

Collaboration with the Michael J. Fox Foundation to advance LRRK2 kinase inhibitors for PD: Areas of focus

**LRRK2 biology**
- **MLi-2**

**Therapeutic Development**
- **MLi-2**

**Enabling Clinical Trials**
- **MJFF LRRK2 Strategy**
  - **Therapeutic Index (mouse)**
    - Inclusion of MLi-2 and MSD in-diet dosing protocol to generate lung phenotype in mice combined with CRL AAV αSynuclein model.
  - **Therapeutic Index (NHP)**
    - AAV αSynuclein model in NHPs; MSD funding for imaging endpoints in the PFE-360 study run at Atuka.
  - **LRRK2 PBMC Consortium**
    - MJFF supported access to LRRK2 G2019S mutation carrier PBMCs in Phase 3 of the PBMC consortium.
  - **MJFF MTAC**
    - MSD participation of the MJFF Mobile Technology Advisory Committee.

**Substrate identification**
- Provision of MLi-2 to the (LEAPS Substrate Initiative results in identification of Rab GTPases.

**De-risking the lung phenotype**
- MLi-2 included in the LRRK2 Safety Initiative which demonstrated reversibility and lack of functional consequences of the phenotype.

**Rab Phos-tag assay**
- MJFF support for Fede Diaz 1 month visit with MRL Boston to develop the phos-tag assay.

**Evaluation of pRab Abs**
- MSD testing of MJFF/Dundee pRab antibodies in LSI tissues and other relevant samples.

**Lung on a chip (Emulate)**
- Levaraging Emulates lung on a chip platform to evaluate the effect of LRRK2 kinase inhibitors in-vitro.
Sharing of MLi-2 with MJFF LEAPS initiative enables identification of bona fide LRRK2 substrates

Phosphoproteomics reveals that Parkinson’s disease kinase LRRK2 regulates a subset of Rab GTPases” Steger et al., eLife 2016, 5:e12813.
Functional interplay between Rab GTPases and several Parkinson’s factors

**Neuron. 2013 Feb 6;77(3):425-39**

*RAB7L1 Interacts with LRRK2 to Modify Intraneuronal Protein Sorting and Parkinson’s Disease Risk*


*Mutations in RAB39B Cause X-Linked Intellectual Disability and Early-Onset Parkinson Disease with α-Synuclein Pathology*

**Science 2006 July 21; 313 (5785):324-8**

*α-Synuclein Blocks ER-Golgi Traffic and Rab1 Rescues Neuron Loss in Parkinson’s Models*

**Proc Natl Acad Sci. 2008 Jan 8;105(1):145-50**

*The Parkinson’s disease protein α-synuclein disrupts cellular Rab homeostasis*

**EMBO J. 2015 Nov 12;34(22):2840-61**

*Phosphoproteomic screening identifies Rab GTPases as novel downstream targets of PINK1*
**Model:** Recombinant α-Syn preformed fibrils (PFFs) seed recruitment of endogenous α-Syn into **insoluble, phosphorylated α-Syn aggregates** (pathological features observed in PD).

![Diagram showing PFF recruitment and aggregation]

Collect Lysates/Insoluble Fraction
Endpoints: Total & Phospho α-Syn

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**α-Syn in Neurons vs. PD Brain**

**PFF Neuron**
- PBS
- PFF
- PFF

**PD Brain**
- Ctrl
- PD
- PD

![Electrophoresis images showing α-Syn aggregates and Monomer]

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**LRRK2 Inhibitors Reduce PFF Formation**

Volpicelli-Daley et al. Neuron 2011
Potential safety concern for LRRK2 Kinase inhibitors

Wild-type LRRK2 KO
Herzig et al., 2011

Pharmacological inhibition of LRRK2 induces a lung phenotype in NHPs

Fuji et al., 2015
MJFF established an unprecedented collaboration of major drug makers willing to collaborate to address key questions about the safety of LRRK2 kinase inhibitors.

Different structural classes of inhibitors with different off-target kinase profiles:

- **GNE-7915**
  - pS935 \textit{in vivo} IC$_{50}$ unbound brain = 97 nM

- **PFE-360**
  - pS935 \textit{in vivo} IC$_{50}$ unbound brain = 3 nM

- **MLi-2**
  - pS935 \textit{in vivo} IC$_{50}$ unbound brain = 0.8 nM
LSI Study highlights

- Mild accumulation of lamellar bodies observed in type II pneumocytes in NHPs; PFE-360 and MLi-2 induced lung histologic effects only at high doses; GNE findings reversible on washout.
- No functionally significant alterations in any pulmonary functional endpoint examined with MLi-2; histology findings were reversible after washout.

Posted online August 15, 2018: doi: http://dx.doi.org/10.1101/390815
LRRK2 kinase inhibition as a genetically supported target for disease modification in Parkinson’s disease

Genetic variants increase risk for sporadic PD

LRRK2 mutations can cause PD
- 5% of familial PD
- 2% of sporadic PD

Clinical and pathological phenotype resembles idiopathic PD

Mutations increase kinase activity
- (G2019S, R1441, I2020T)

LRRK2 Therapeutic Rationale

Biology converging on PD-relevant pathways:
- Linkage to α-Synuclein pathology

Inhibition of LRRK2 kinase activity represents a tractable and novel therapeutic target to modify the progression of Parkinson’s disease
Acknowledgments

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- LRRK2 TEAM