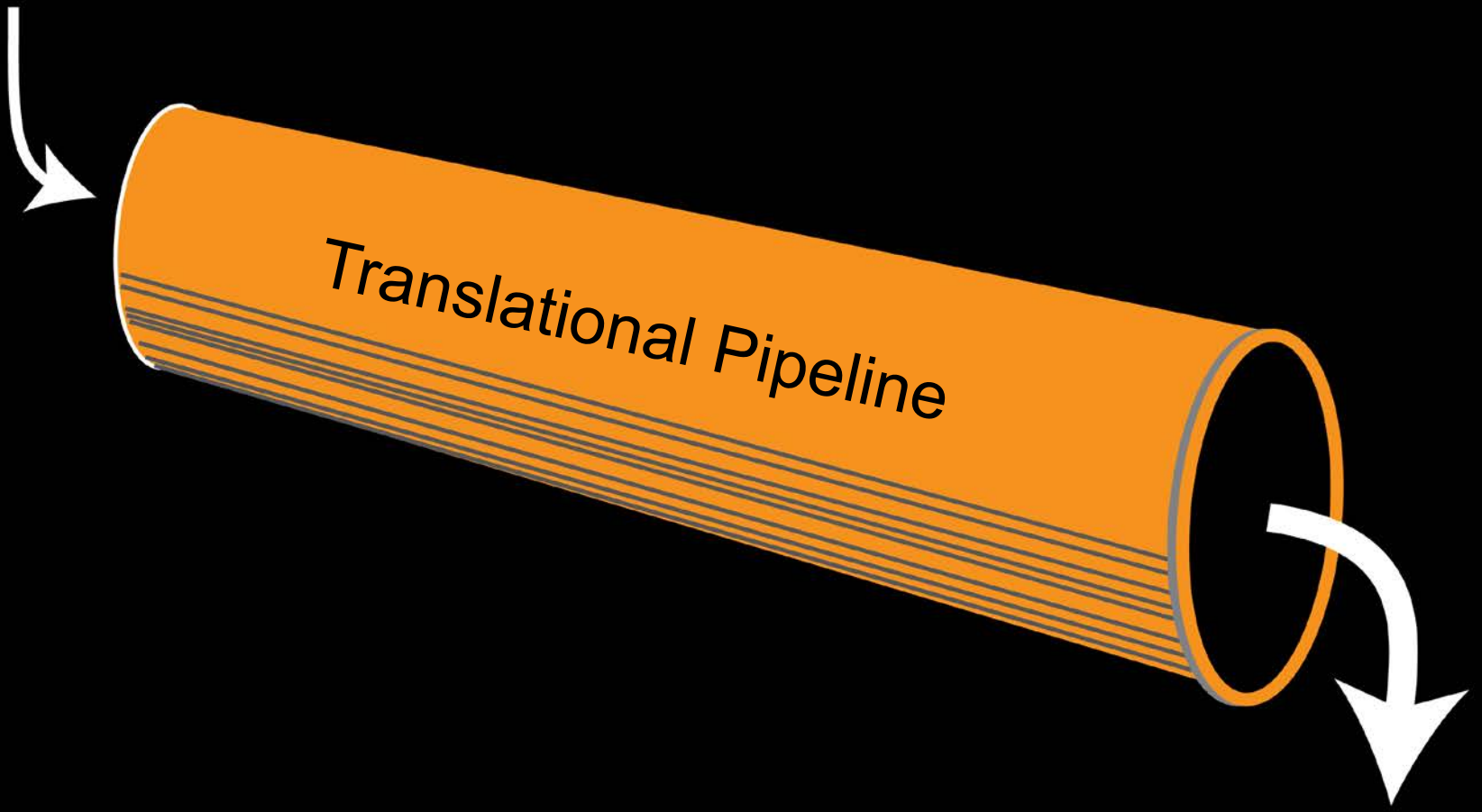
Introduction to Session 2: Building a Translational Pipeline for Parkinson's Disease Therapeutics

Co-Chairs: John Dunlop, PhD and
Steven Finkbeiner, MD, PhD

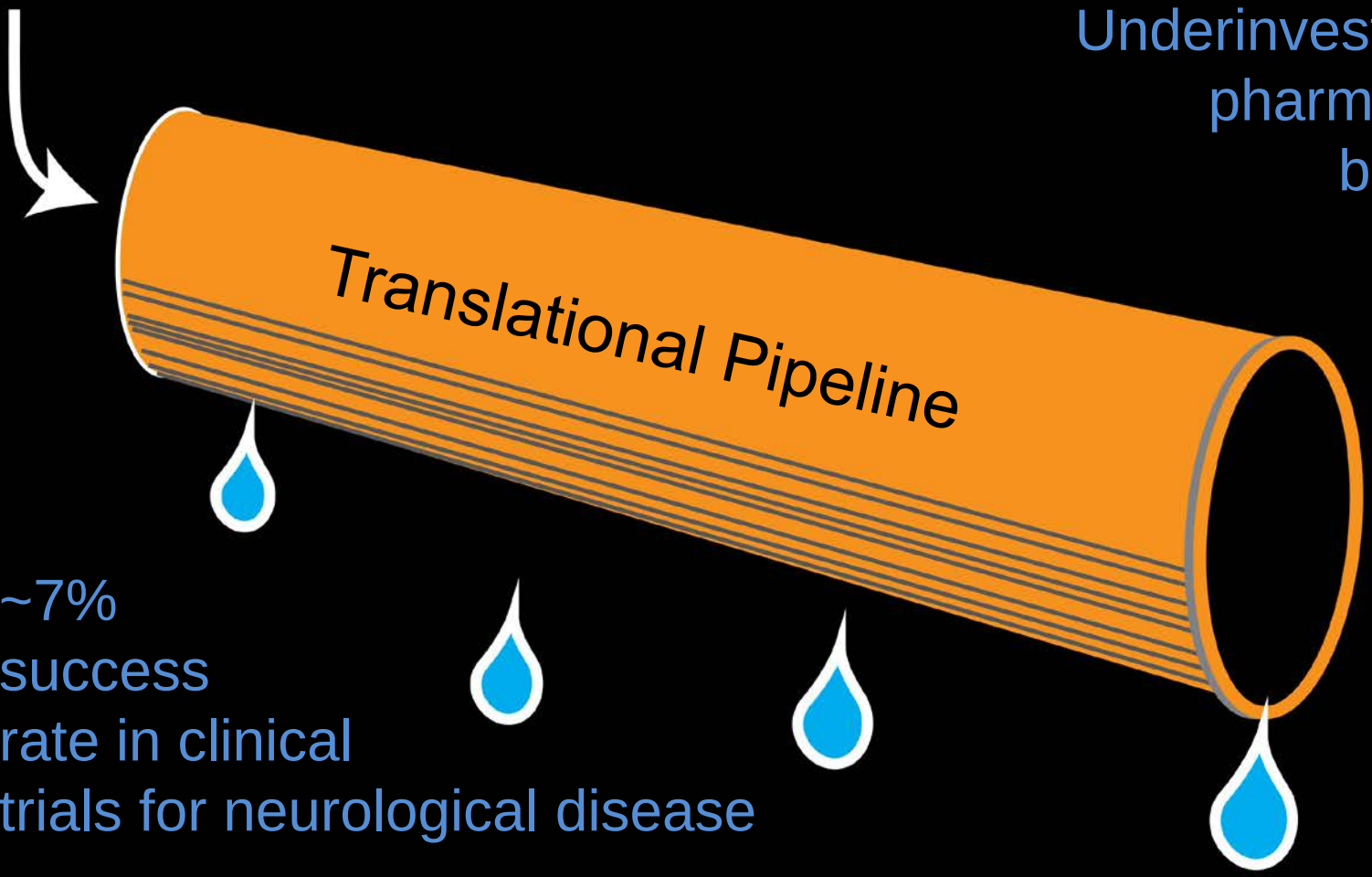
NIH Liaisons: Margaret Sutherland, PhD,
and Hao Wang. PhD.

Basic Science Discoveries in Parkinson's Disease



Effective Medicines for People with Parkinson's Disease

Basic Science Discoveries in Parkinson's Disease



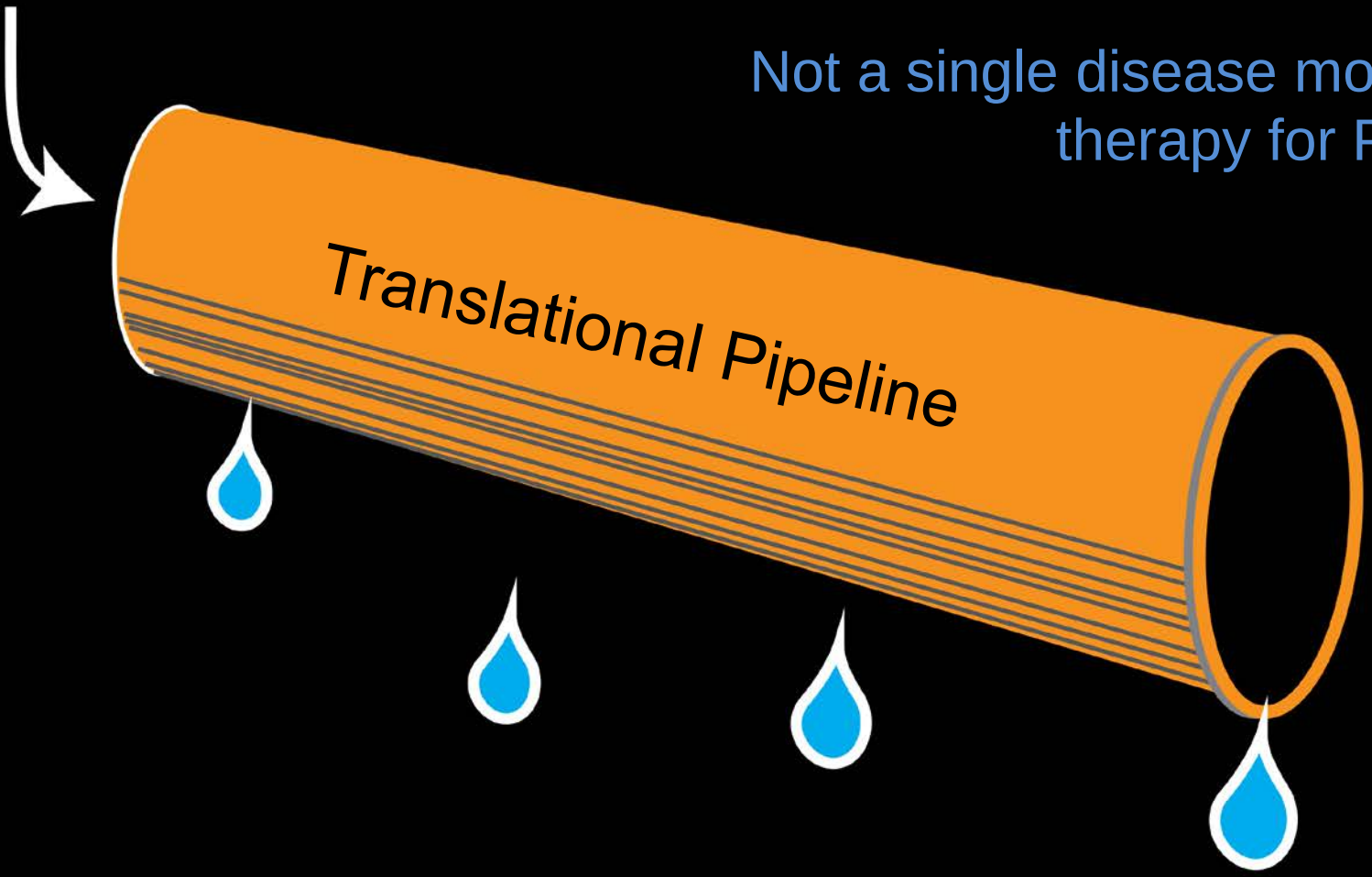
Underinvestment by pharma 2-10X based on societal burden

~7% success rate in clinical trials for neurological disease

Effective Medicines for People with Parkinson's Disease

Basic Science Discoveries in Parkinson's Disease

Not a single disease modifying therapy for PD yet!



Effective Medicines for People with Parkinson's Disease

Recommendations: People and Process

People

- Rudi Balling
- John Dunlop
- Steven Finkbeiner
- John Hardy
- Wade Harper
- Dean Jones
- Hartmuth Kolb
- Dimitri Krainc
- Michael Lee
- Kalpana Merchant
- Clive Svendsen
- Richard Youle

Process

- Brainstorming session to assemble a committee with critical expertise
- Series of teleconferences to formulate, debate, and refine proposed recommendations
- Distill, coalesce, and rank the 10 major recommendations from the group.
- Divide the recommendations into 4 topic areas for this meeting

Recommendations: People and Process

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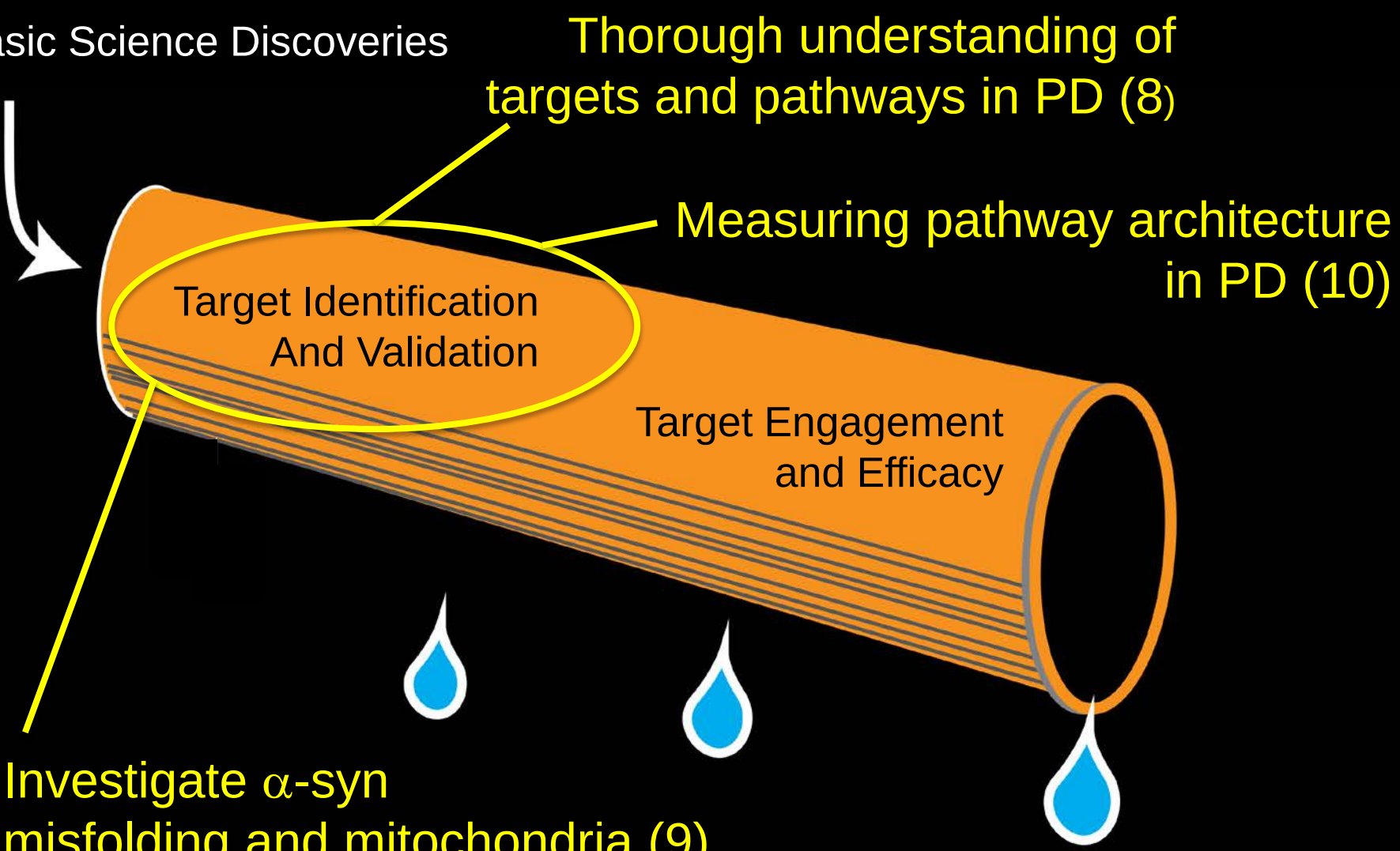
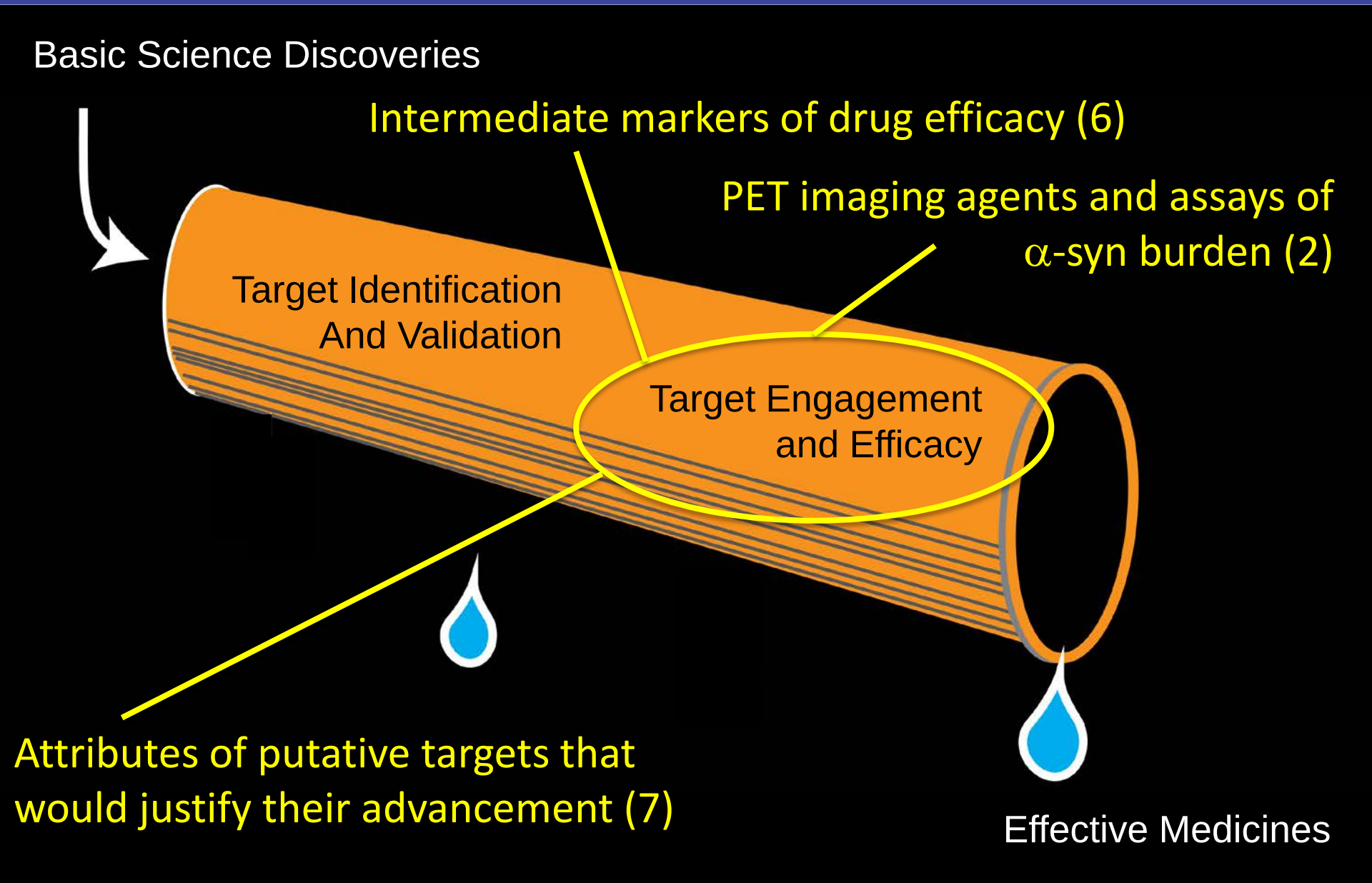
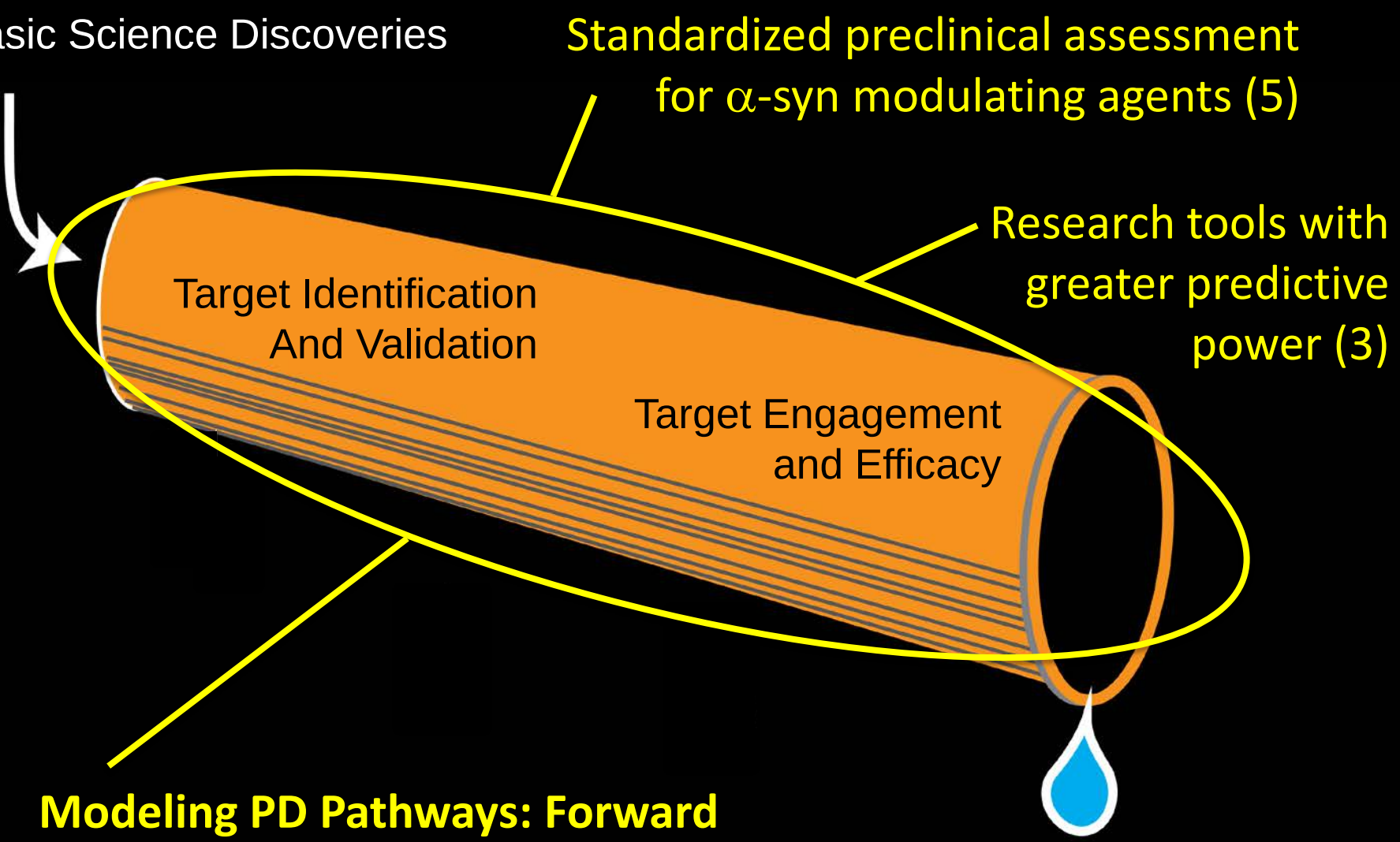

Basic Science Discoveries
Thorough understanding of targets and pathways in PD (8)
Measuring pathway architecture in PD (10)
Target Identification And Validation
Target Engagement and Efficacy
Investigate α-syn misfolding and mitochondria (9)
Effective Medicines


Basic Science Discoveries
Intermediate markers of drug efficacy (6)
PET imaging agents and assays of α-syn burden (2)
Target Identification And Validation
Target Engagement and Efficacy
Attributes of putative targets that would justify their advancement (7)
Effective Medicines


Basic Science Discoveries
Standardized preclinical assessment for α-syn modulating agents (5)
Research tools with greater predictive power (3)
Target Identification And Validation
Target Engagement and Efficacy
Modeling PD Pathways: Forward and Reverse Translation
Effective Medicines

Basic Science Discoveries

Patient stratification Strategies (1)

Integrated PD Databases (4)

Effective Medicines

Session 2 – Building a Translational Pipeline for Parkinson's Disease Therapeutics

Patient/Disease Stratification

John Dunlop

AstraZeneca

Neuroscience Drug Discovery and Development Presents Major Challenges

Scientific challenges	• Biological complexity and un-validated targets • Poor pre-clinical models • Challenge of the blood-brain-barrier • Direct examination of drug exposure and target engagement
Clinical challenges	• Patient recruitment • Patient heterogeneity • Disease is advanced when symptoms appear • Capturing therapeutic effects on clinical scales with high variability
Low productivity	• Long cycle times • High costs • Low probability of success

Parkinson's Disease 2014: Advancing Research, Improving Lives

Biology – Moving Toward Innovative Treatments

Building a Translational Pipeline

Bridging Emerging Science Through Clinical Research

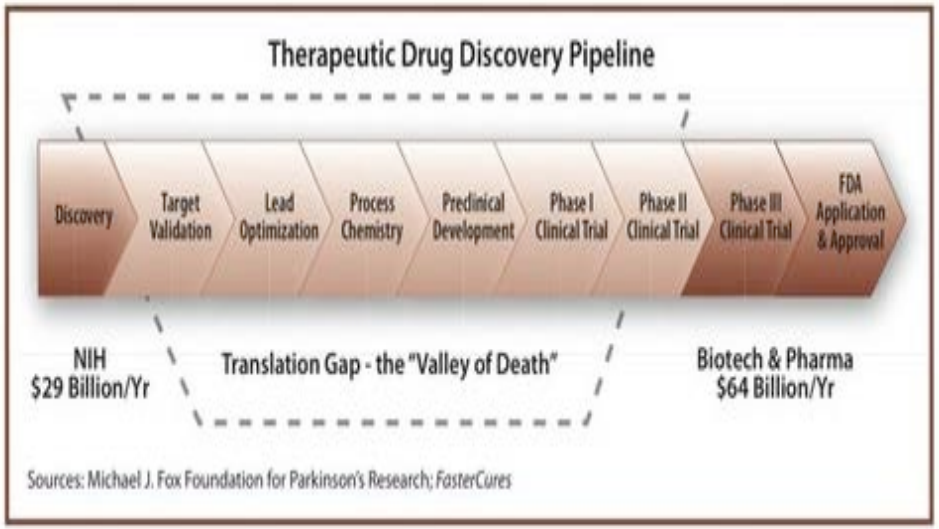
Integrate Thinking and Investment Horizontally

Patient & Disease Stratification

1. Develop patient stratification strategies and support technologies that define disease signatures that represent more homogeneous cohorts for translational research. To stratify PD patients, objectively, disease signatures should define slow and fast progressing PD, prodromal PD and non-motor symptoms of disease.

4. Support the development of an integrated PD database that includes data from genetic, biomarker, clinical research and clinical trials with informatic support for integration of existing databases in PD, and other chronic neurodegenerative diseases.

Translational Valley of Death - Where Do The Challenges Lie?



Answer = Everywhere

Target Selection	Target Screening	Target Validation	Safety, Tox	Clinical Program
'abstract'	**'forced'**	**'rats and mice'**	**'standard'**	**'cookie cutter'**

Where is This Taking Us?

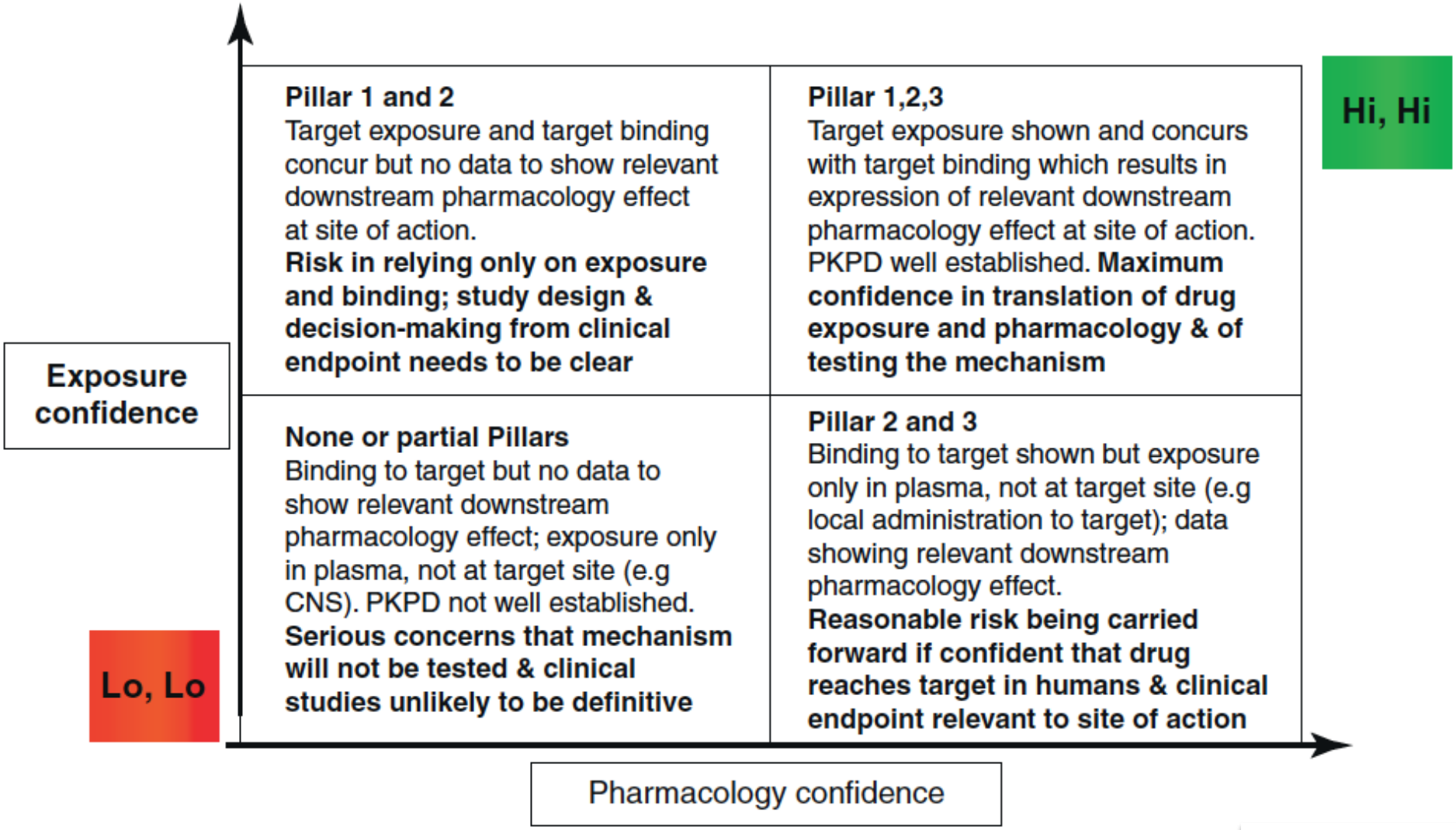
Before	After
- Target ID based on animal data	-Target ID requires human data
- Screens done quickly and without thought	-Thoughtful, relevant (iPS, biased signaling)
- Behavioral endpoints dominated	-Ephys circuit based, histological endpoints
- Normal animals used	-Tg, lesion animals used
- Acute dosing	- Dosing regimens explored
-Target engagement optional	-Target engagement obligatory
- Mechanism unknown	- Mechanism explored
- Knowledge in patient population optional	- Biology of patient explored
-Walk away after failed Ph2	- Repeat your expts

Three Pillars of (Drug) Survival[1]

- **Drug exposure at the target site of action**
  - Drug exposure at the target site of action is necessary to elicit a pharmacological effect over time
- **Binding to the pharmacological target**
  - Target occupancy is a prerequisite for expression of pharmacology and target modulation
- **Expression of pharmacology**
  - Functional modulation of the target is a prerequisite for expression and pharmacological activity in order to test the mechanism

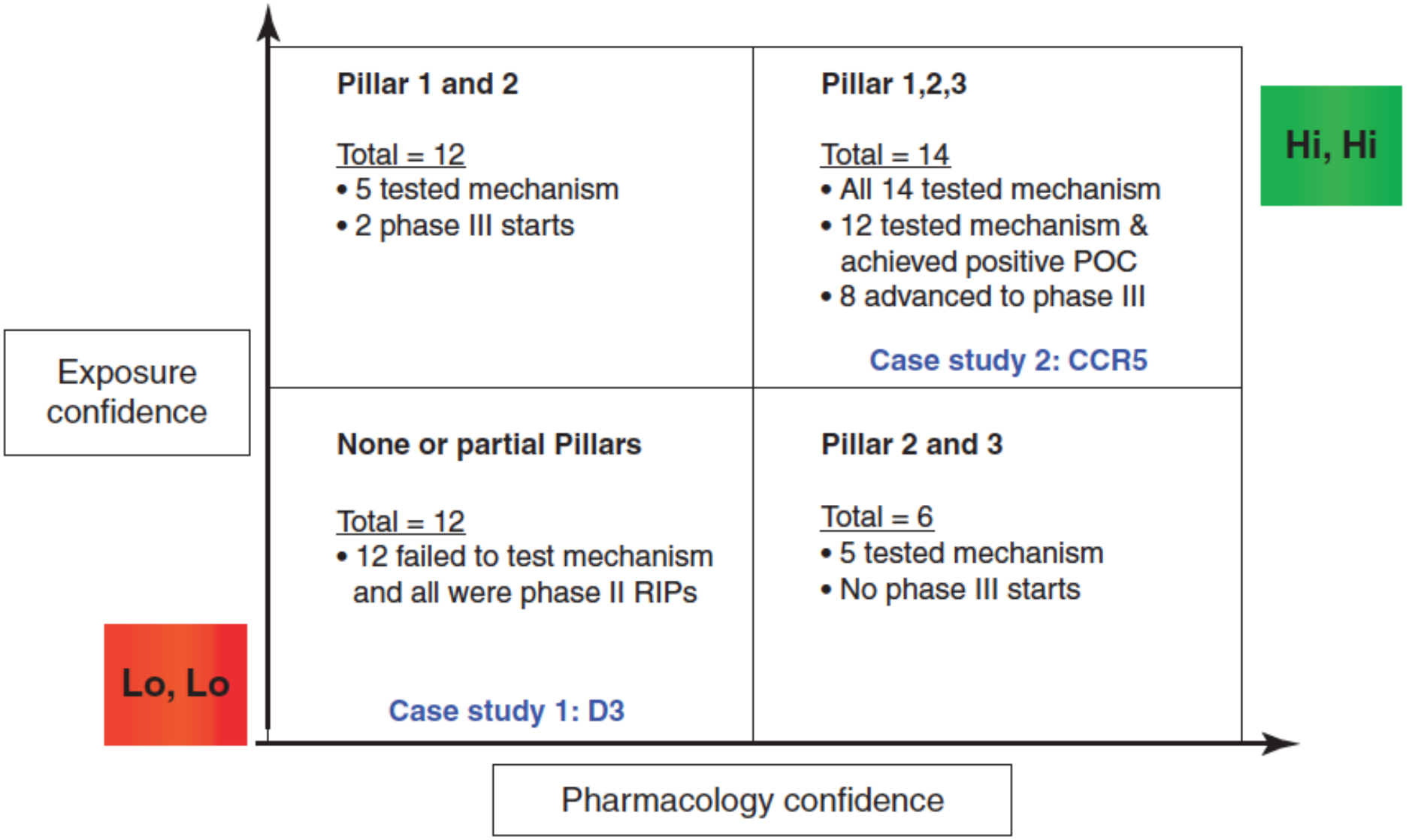
1. Morgan et al., Drug Discovery Today 17, 419-424, 2012

Building Confidence in Pharmacology and that the Proposed Mechanism will be Tested



From Morgan et al., Drug Discovery Today 17, 419-424, 2012

Impact of 3 Pillars on 44 PFE Programs Between 2005-2009



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Challenges of Patient Selection in Parkinson's Disease

- Inclusion of patients who have Parkinson's disease
- Identifying patients with the highest probability of benefiting from the mechanism of action associated with the intended therapeutic

Challenges of Patient Selection in Parkinson's Disease

- Inclusion of patients who have Parkinson's disease
  - Possible dilution of clinical effect size due to enrolled patients who do not have the disease
  - Lessons from Phase 3 AD trials –
    - Amyloid imaging in bapineuzumab trials revealed 36.1% in ApoE4 non-carriers and 6.5% in ApoE carriers had signals below predetermined threshold for amyloid positivity[1]
    - Similarly 32.8% of patients enrolled in solanezumab Ph3 study were below threshold of amyloid positivity[2]

1. Vellas et al., 2013, Alzheimer's and Dementia 9, 438-444
2. Siemers E, personal communication, CTAD Task Force, Nov 13 2013, San Diego

Synuclein Imaging as a Critical Need

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DOI 10.3233/JPD-130247
IOS Press

α-synuclein Imaging: A Critical Need for Parkinson's Disease Research

Jamie L. Eberling*, Kuldip D. Dave and Mark A. Frasier
The Michael J. Fox Foundation for Parkinson's Research, New York, NY, USA

Abstract. The development of an α-synuclein imaging agent could be transformative for Parkinson's disease research and drug development. The ability to image α-synuclein in the brain would enable tracking of the degree and location of pathology over time and monitoring of therapies aimed at reducing α-synuclein levels. The Michael J. Fox Foundation has assembled a consortium of researchers to develop an α-synuclein radiotracer for use in positron emission tomography (PET) imaging studies. While this poses a number of challenges they should not be insurmountable and lessons learned from the development of tau radiotracers should provide valuable insights.

Keywords: α-synuclein, positron emission tomography, radiopharmaceutical, biomarker, β-amyloid, tau

Patient Stratification in Parkinson's Disease

- Identifying patients with the highest probability of benefiting from the mechanism of action associated with the intended therapeutic

  - Identify the most promising biomarkers for patient stratification
  - Develop sensitive imaging and biofluid assays for alpha-synuclein to assess Lewy body pathology and soluble species of alpha-synuclein, respectively
  - Develop novel genetic and genomic markers, markers of neuronal health and synaptic proteins
  - Develop an understanding of the factors contributing to heterogeneity in the rate of progression of Parkinson's disease

Patient & Disease Stratification

1. Develop patient stratification strategies and support technologies that define disease signatures that represent more homogeneous cohorts for translational research. To stratify PD patients, objectively, disease signatures should define slow and fast progressing PD, prodromal PD and non-motor symptoms of disease.

4. Support the development of an integrated PD database that includes data from genetic, biomarker, clinical research and clinical trials with informatic support for integration of existing databases in PD, and other chronic neurodegenerative diseases.

Integrated Parkinson's Disease Database

Need: An enabling approach would be to develop PD signatures by collecting high resolution molecular data (e.g., whole genome sequencing, transcriptomics, epigenomics, metabolomics, proteomics, iPSC phenotypic data, etc) and apply systems approaches to determine whether signatures of PD emerge that have important predictive value for clinical features, such as onset, progression, symptom profile and/or to identify new pathways and targets implicated in the pathophysiology of the disease. It is anticipated that disease signatures developed at different times would reveal whether the abnormalities found in patients cluster into a single group or multiple groups. Samples from clinical trials in PD should also be mined using the approaches described above to determine the extent to which responses among different PD patients are homogeneous or heterogeneous.

Approaches:

- Deploy systems approaches (omics) on their own and in combination, especially using human information to develop an understanding of disease and effects of perturbations.

- Develop computational tools such as machine learning and other approaches to integrate disparate types of data into a global expression of disease signatures and to investigate the prognostic value of multidimensional data sets for disease progression (now) and response to therapy (longer term).

- Deploy funds in support of open access databases and shared resources

Panel Discussion

Panel Discussion - Patient & Disease Stratification

Panelists – Rudi Balling, Steve Finkbeiner, John Hardy, Dean Jones; Moderator – John Dunlop

1. Develop patient stratification strategies and support technologies that define disease signatures that represent more homogeneous cohorts for translational research. To stratify PD patients, objectively, disease signatures should define slow and fast progressing PD, prodromal PD and non-motor symptoms of disease.

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Session 2- Building a Translational Pipeline for Parkinson's Disease Therapeutics

Target Engagement and Efficacy

Dimitri Krainc

Recommendation: Define the required attributes of targets emerging from basic science efforts that would justify their advancement into translational studies in Parkinson's disease

Consider targets that are already "validated" in patients via genetic and clinical linkages to develop **targeted and personalized therapies.**

Example 1

Enzyme X that modulates levels of alpha-synuclein (e.g. glucocerebrosidase):

- -Establish in-depth mechanistic link between X and a-syn
- -Activate X with small molecule (e.g. via direct binding to X)
- -Assess X activity in accessible peripheral compartments (e.g. CSF, blood)
- -Determine if activation of X lowers a-syn in CNS--link to peripheral a-syn

Example 2

Therapeutic agent that partially rescues survival and/or neuropath in PD models (e.g. antioxidants, coenzymeQ, etc)

- -Specific target not known or mechanistic link not established
- -Target engagement cannot be assessed in models or patients
- -Expensive trials required to evaluate effects in patients

Development of Targeted Therapies Improves Healthcare—examples from oncology

	Description	Examples
Earlier detection	Molecular testing enables diagnosis before patient presents with phenotype	▪ BRCA 1/2 testing in breast cancer ▪ CDKN2A/2B SNP test for MI risk ▪ Prenatal gene testing
Disease categorization	Patients with the same diagnosis are categorized according to molecular signatures for more effective therapy	▪ HER2 testing in breast cancer ▪ CCR5 and CXCR4 co-receptor in HIV ▪ BCR-ABL testing in leukemia
Choice/dose of therapy	Specific drug and dose selected to minimize side effects and maximize patient response on the basis of an individual's molecular profile	▪ VKORC1/CYP2C9 for warfarin ▪ TMPT test in leukemia ▪ UGT1A1 test in colorectal cancer

Recommendation: Develop specific α-Synuclein PET imaging agents and assays to measure alpha synuclein burden, validated in both animal models and human tissue.

Non-invasive confirmation of alpha-synuclein pathology is critical to support the accuracy of clinical diagnosis and can be used in combination with CSF measures of alpha-synuclein, to track the temporal profile of disease progression and to monitor the effect of therapies that alter alpha-synuclein levels and species.

Examples:

Develop selective and potent α-synuclein tracers by following structure-activity trends, maximizing α-synuclein selectivity and minimizing binding to other amyloid proteins.

α-synuclein, being a pathological hallmark of PD may serve as a diagnostic target for PD diagnosis and for monitoring disease progression.

Validated and standardized assay methodologies are needed to measure soluble, oligomeric and post-translational modified forms of alpha-synuclein in plasma and CSF.

Recommendation: Develop intermediate markers of drug efficacy in early Parkinson's disease translational studies to support more cost-effective and smaller proof-of-concept studies.

Examples:

Small molecule activates enzyme X (e.g. CSF, brain) and lowers a-synuclein in patients.

What to measure next?

Neuroimaging biomarkers (e.g. DAT scans, DTI, connectivity maps with BOLD-MRI, etc) to assess their utility in shorter-term (e.g., 6 months) trials.

Neurophysiology markers such as beta oscillations assessed by EEG/MEG

Protein flux using CSF proteomics approaches

Turnover rate of alpha-synuclein at different stages of PD (e.g. leucine labeling)

Summary

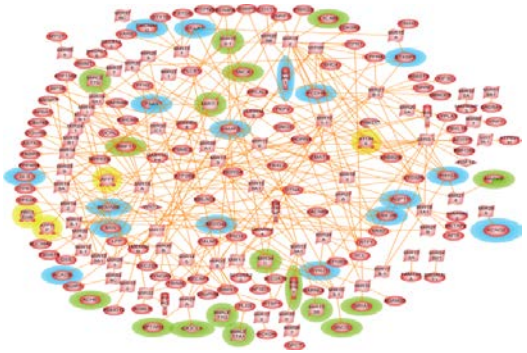
Define the required attributes of targets emerging from basic science efforts that would justify their advancement into translational studies in PD.

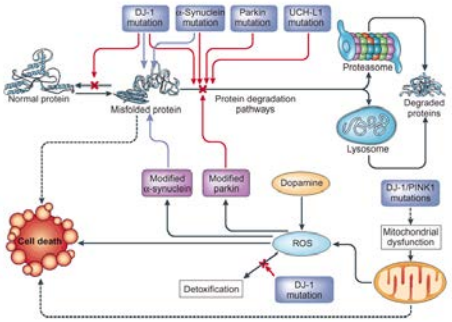
Develop and apply intermediate markers of drug efficacy in early PD translational studies to support more cost-effective and smaller proof-of-concept studies.

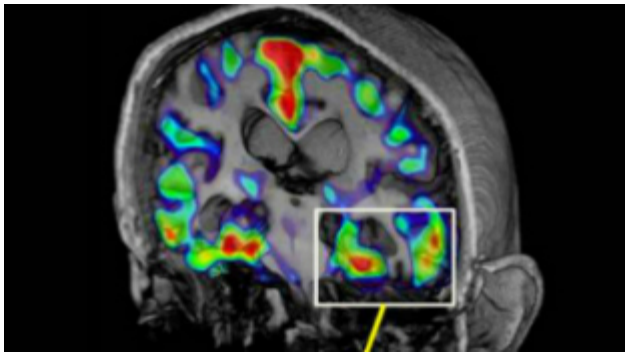
Develop specific α-Synuclein PET imaging agents and assays to measure alpha synuclein burden

Invest in "input" stage of the translational process– e.g. targets and pathways

"Low input---high throughput---no output" (Sydney Brenner)







Panel Discussion

Target Engagement and Efficacy

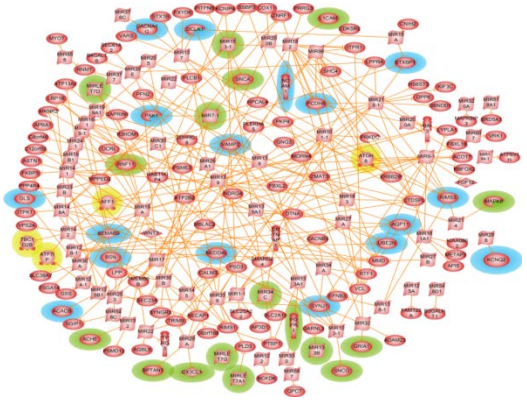
Panelists - Dimitri Krainc, John Hardy, Richard Youle, John Dunlop

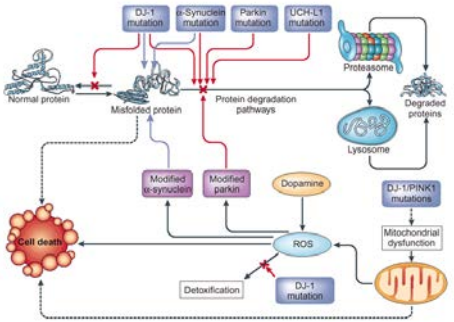
Moderator – Steve Finkbeiner

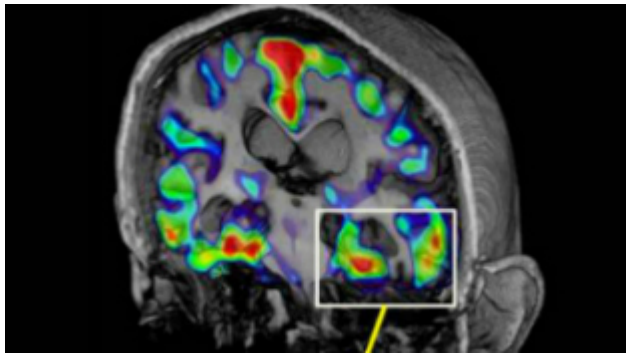
2. Develop novel and specific α-Synuclein PET imaging agents and assays to measure alpha synuclein burden, validated in both animal models and human tissue.

6. Develop and apply intermediate markers of drug efficacy in early Parkinson's disease translational studies to support more cost-effective and smaller proof-of-concept studies.

7. Define the required attributes of targets emerging from basic science efforts that would justify their advancement into translational studies in Parkinson's disease.







Session 2- Building a Translational Pipeline for Parkinson's Disease Therapeutics

Modeling PD Pathways

Mike Lee and Clive Svendsen

Modeling PD Pathways

5. For preclinical studies targeting alpha-synuclein metabolism, alpha-synuclein pathology and/or neurodegeneration caused by alpha-synuclein, procedures and models used should be standardized, with consensus guidelines developed for appropriate use of existing models, replication of results across labs, and recommendations for future model development.

3. Support the development of translational resources with greater power to predict efficacy and biomarker outcomes in clinical trials. These resources would include well- characterized, replication sets of non-integrating iPSC lines from sporadic cases and from patients with each major PD-causing gene mutation.

Modeling PD Pathways

Recommendation 5: For preclinical studies targeting alpha-synuclein pathology, procedures and models used should be standardized, with consensus guidelines developed for appropriate use of existing models and recommendations for future model development.

Need: Using a standard set of models and procedures will facilitate direct comparison of various preclinical studies within labs and across different labs and treatment options. Even for the alpha-synuclein based models, there is a confusing array of possible models with very different outcome measures.

Approaches:

- Identify a few models that might be "predictive" for clinical trials and appropriate outcome measures that should be used.
- If non-standard models are used, define appropriate outcome measures for targeting alpha-synuclein.
- Determine if alpha-synuclein causes common abnormalities in different models (transgenic, inoculation-spreading, virus-induced, and cell based).
- Provide standardized collection of pathological tissues from animal models and human cases for unbiased pathway/omics analysis
- Organize NINDS sponsored workshop/panel to develop consensus guidelines for preclinical use of animal models for the assessment of *in vivo* assays. These guidelines should be reviewed regularly and modified.

Why Focus on alpha-synuclein pathology?

Mutations in alpha-synuclein gene recapitulates progressive and neuropathological features of PD

Sporadic (~90%)

- Causes are unknown,
- Risk factors (relatively weak)
  Environment, Pesticides/metals
  α-Syn gene(SNCA) polymorphism
  GBA (glucocerebrosidease)
  mutation
- Protective factors (Nicotine, caffeine)
- Pathology

LewyBodies/α-syn aggregates
SNpc and extra-SN degeneration
Progressive, common non-motoric abnormalities, dementia.

≈

≠

Hereditary/Genetic (~10%)

Autosomal Dominant

- *α-Syn* gene mutation/multiplication
- *LRRK2* mutations, most common. Late onset partial penetrance
- Pathology

Lewy Bodies, α-syn aggregates, SNpc and extra-SN degeneration
Progressive, common non-motoric abnormalities, dementia.

Autosomal Recessive

- Loss of function
- *PRKN, PINK1, DJ-1* mutations/deletions.
- Pathology

Selective SNpc degeneration, many cases without Lewy body pathology
Slowly Progressive, limited non- motoric abnormalities.

Is Alpha-synuclein pathology key to disease modification?

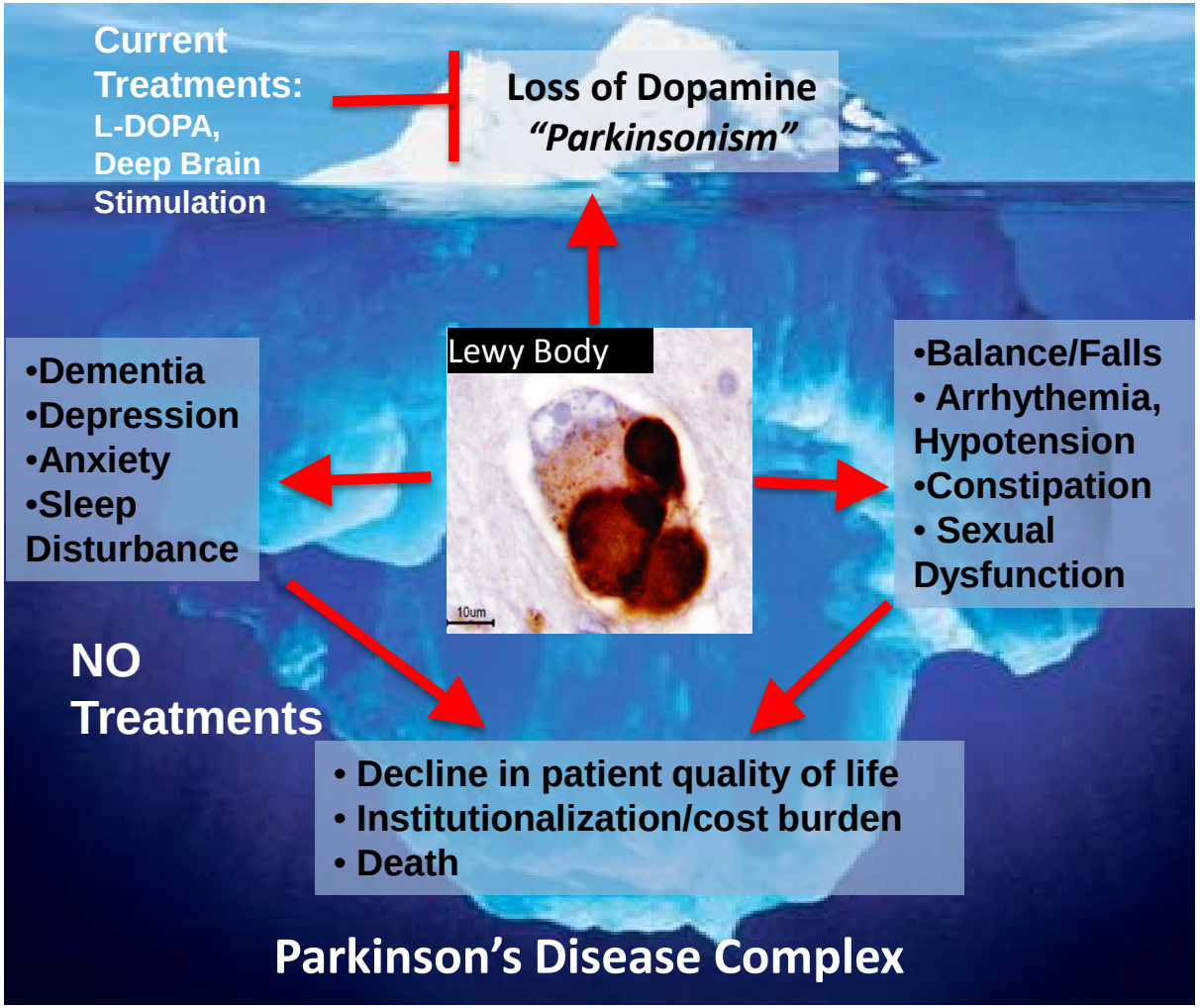
Progressive features of PD may be caused by progressive alpha-synuclein pathology

Models:

- Toxins, MPTP, 6-OHDA, Pesticides
- Genetic, Viral and TH/DAT promoter.

Models:

- Transgenic rodents
  - Pan Neuronal
  - Cell type specific
  - Conditional
- Viral (AAV and Lenti)



Neuroprotection in Toxin Models but failure in Clinical Trials

Agent	Proposed mechanism of action	Outcome measure used
Tocopherol	Lipid-soluble antioxidant	Time prior to need for L-dopa therapy
TCH346	Anti-apoptotic	Time prior to need for L-dopa therapy
Riluzole	Anti-glutamate	Time prior to need for L-dopa therapy
CEP1347	Anti-apoptotic	Time prior to need for L-dopa therapy
Immunophilin	Anti-apoptotic	Change in UPDRS score
CoQ10 and Vitamin E	Anti-oxidant/bioenergetic	Change in UPDRS score
Preladenant	Adenosine A2A receptor antagonist	Change in UPDRS score
Cogane	Promote release of BDNF and GDNF	Change in UPDRS score

Is alpha-synuclein model different then toxin models ?

CINAPS project evaluated preclinical efficacy of Pioglitazone and Isradapine using the mouse MPTP toxicity model and the A53T mutant alpha-synuclein transgenic mouse model (line G2-3).

(http://www.ninds.nih.gov/research/parkinsonsweb/cinaps/index.htm)

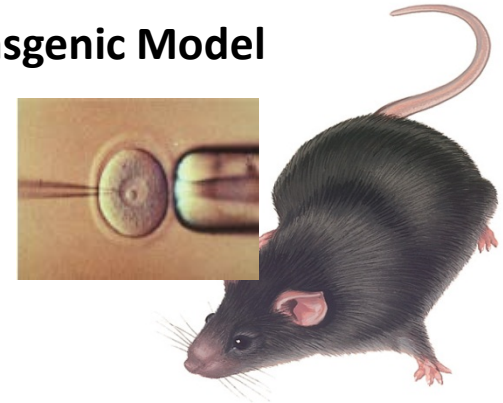
1. **Both Pioglitazone and Isradapine reduce neurodegeneration in Toxin model.**
   - -Prevention protocol
   - -HPLC analysis of Striatal Dopamine
   - -Stereological analysis of SNpc dopamine neurons
2. **Pioglitazone or Isradapine did not delay disease onset or progression in the mutant alpha-synuclein transgenic mouse model**
   - -Prevention protocol, treatment started at 8 mos of age.
   - -Behavioral observation
   - -Neuropathological and biochemical analysis
3. **Does anything delay disease in the alpha-synuclein model?**
   - -Salubrinal, delay average age of disease onset by >4 mos in A53T transgenic mouse model and attenuates neurodegeneration in AAV-alpha-synuclein rat model (Colla et al., J. Neurosci. 2012)
   - -alpha-synuclein immunization (e.g. Prevents Cell-to-Cell transmission, Bae et al., J. Neurosci. 2012)
   - -Nilotinib, attenuates loss of SNpc dopamine neurons in lentiviral model (Hebron et al., HMG, 2013)

Currently, toxin-based models are not predictive for neuroprotection trials. Future trials will determine if alpha-synuclein based models are predictive for neuroprotection trials.

Why do we need standardization ?

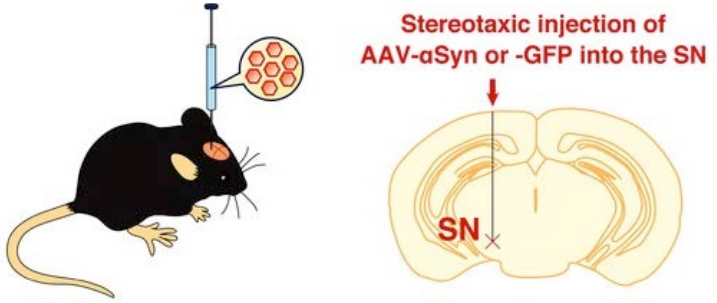
Confusing array models and phenotypes

Transgenic Model



- Species: Mouse, Rat, other
- Pan-Neuronal expression (Prp, Thy1, PDGF)
- Cell type specific expression (TH, DAT, Oligos)
- Endogenous expression (BAC, PAC)
- Inducible/Conditional expression (Tet-O, Flox)
- Phenotypes: Various combinations of alpha-synuclein pathology, behavior, and neurodegeneration

Viral transduction Model



- Species: Rat, Mouse (?), Primates
- AAV
- Lentivirus
- Phenotype: Neurodegeneration, behavior, some synuclein pathology. Neurodegeneration is not consistent.

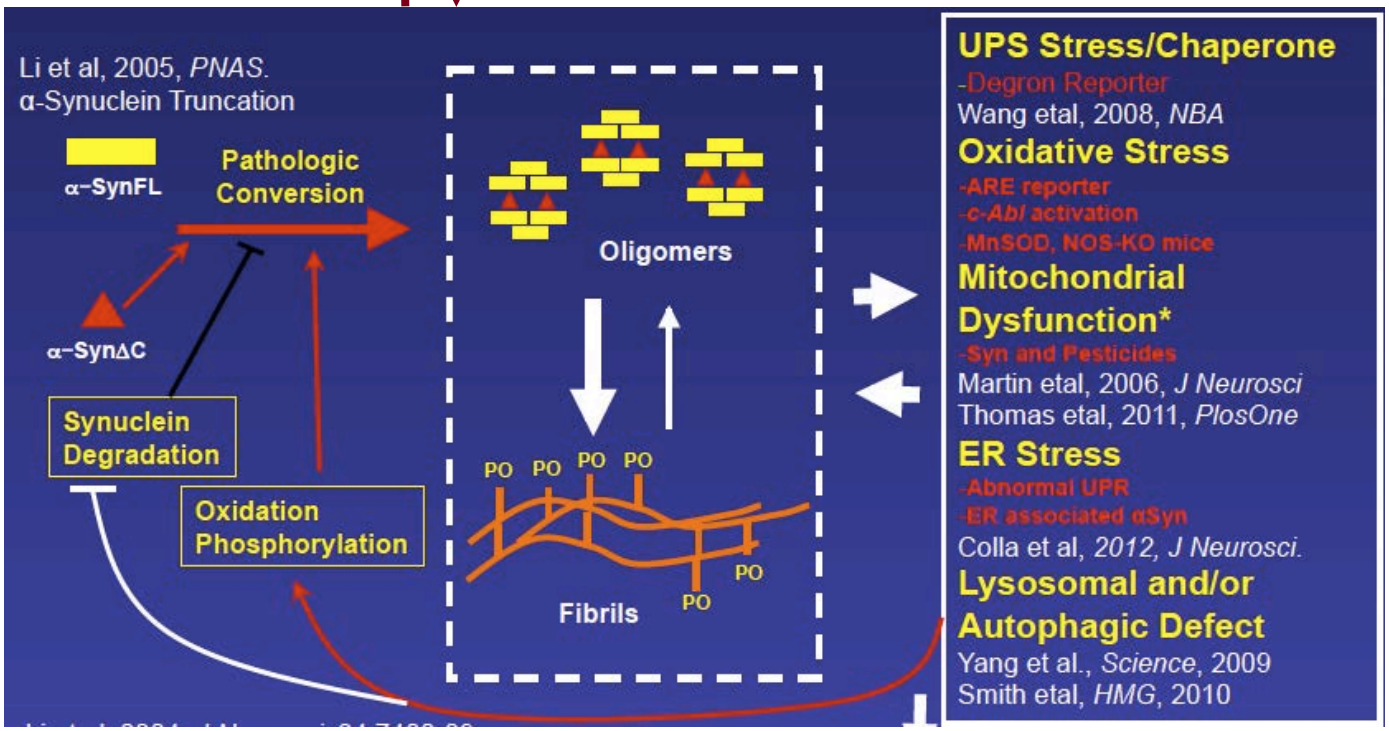
Synuclein-Innoculation/Transmission Model

- Mouse, rat, primate
- Transgenic and nontransgenic animals

Facilitate replication and comparative analysis

What targets can be evaluated in alpha-synuclein based models ?

LRRK2, GBA mutant



Neurodegeneration

Pesticide/Environment/Mitochondria (sporadic)

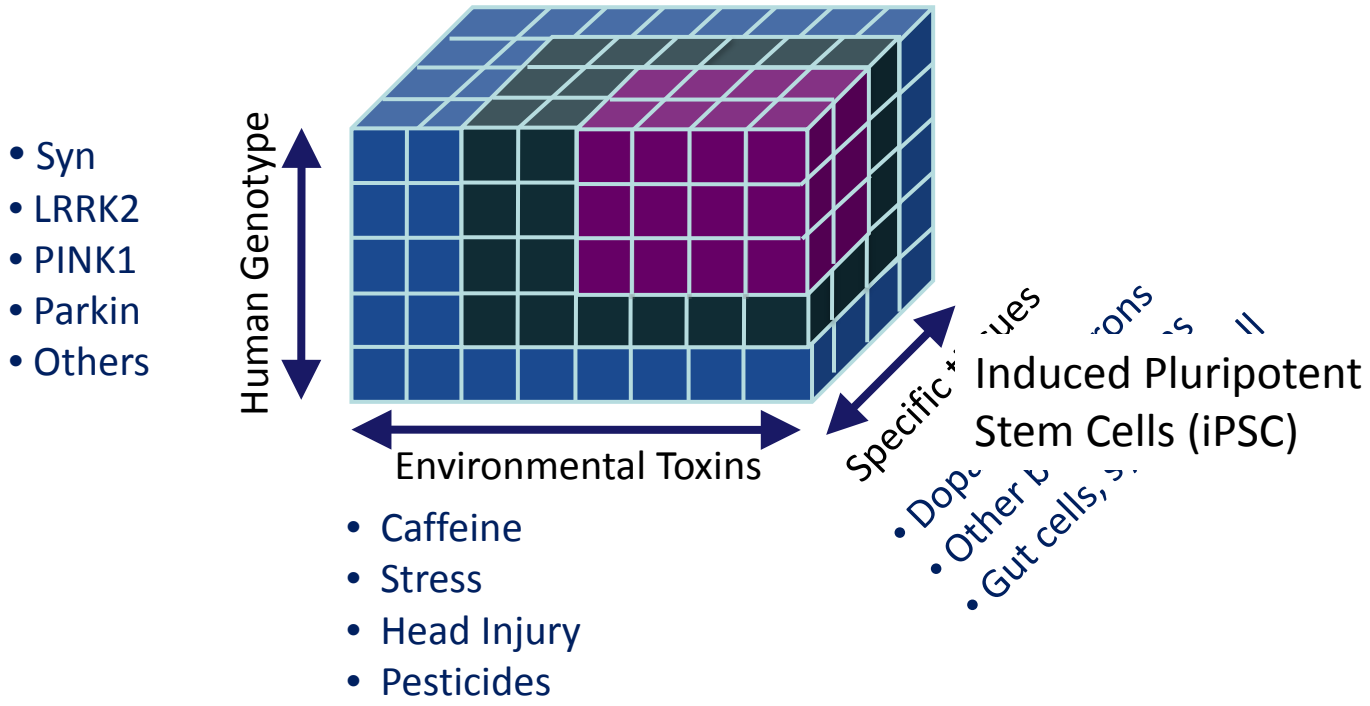
***Parkin can be inactivated by Nitration (Chung etal., *Science*, 2004) and/or Tyr-Phosphorylation by cAbl (Ko etal, *PNAS*, 2010).**

Modeling PD Pathways

5. For preclinical studies targeting alpha-synuclein metabolism, alpha-synuclein pathology and/or neurodegeneration caused by alpha-synuclein, procedures and models used should be standardized, with consensus guidelines developed for appropriate use of existing models, replication of results across labs, and recommendations for future model development.

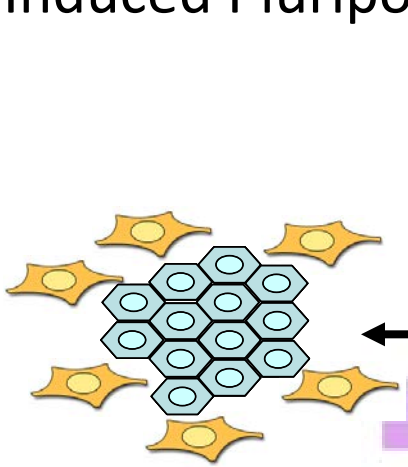
3. Support the development of translational resources with greater power to predict efficacy and biomarker outcomes in clinical trials. These resources would include well- characterized, replication sets of non-integrating iPSC lines from sporadic cases and from patients with each major PD-causing gene mutation.

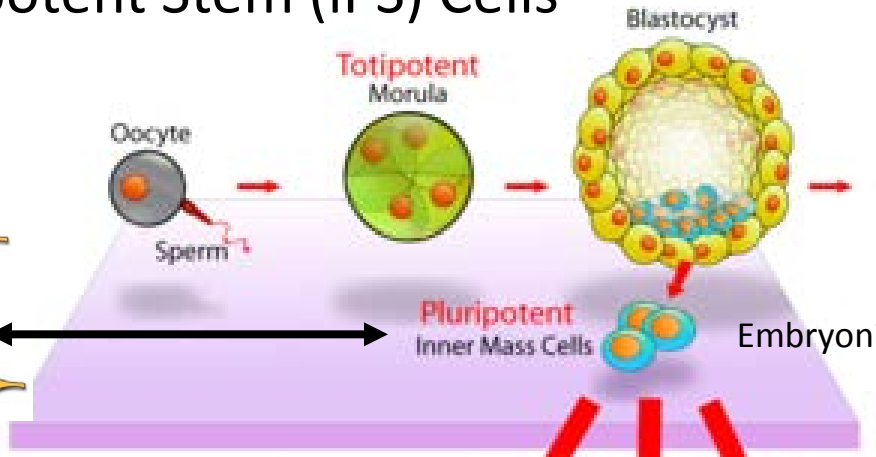
Parkinson's Disease – Modeling Pathways



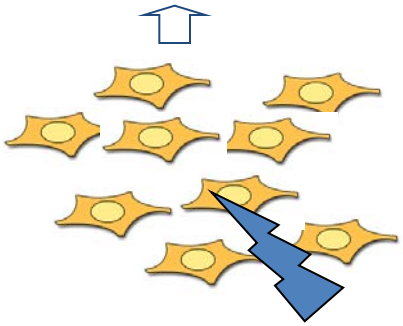
Induced Pluripotent Stem (iPS) Cells

Takahashi et al *Cell* 131: 1-12 (2007)
Yu et al, *Science* 318: 1917-20 (2007)
Park et al, *Nature* 451: 141-46 (2008)



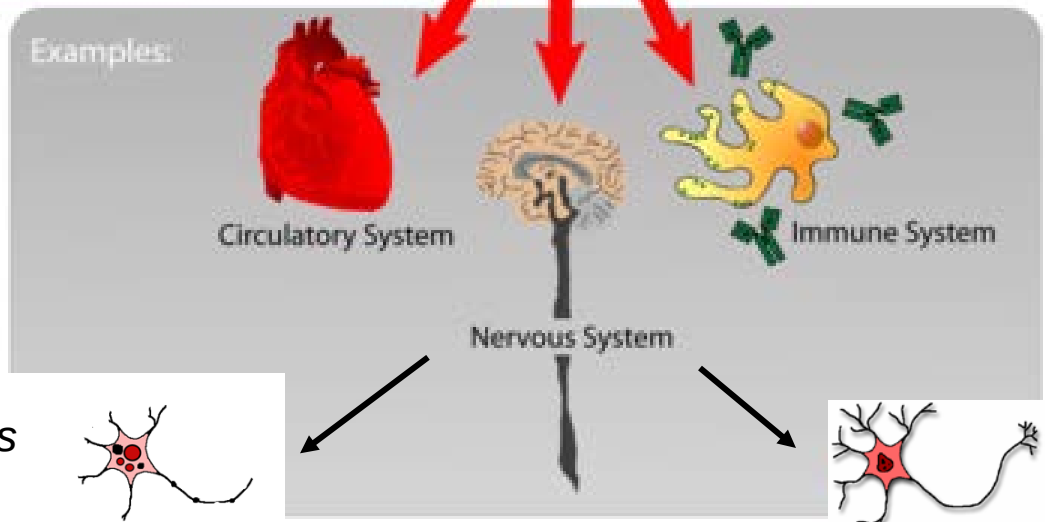


iPS line - pluripotent



+ Oct4, Sox2, others
Reprogram…

Skin cells



Diseased
Neuron

Neuron

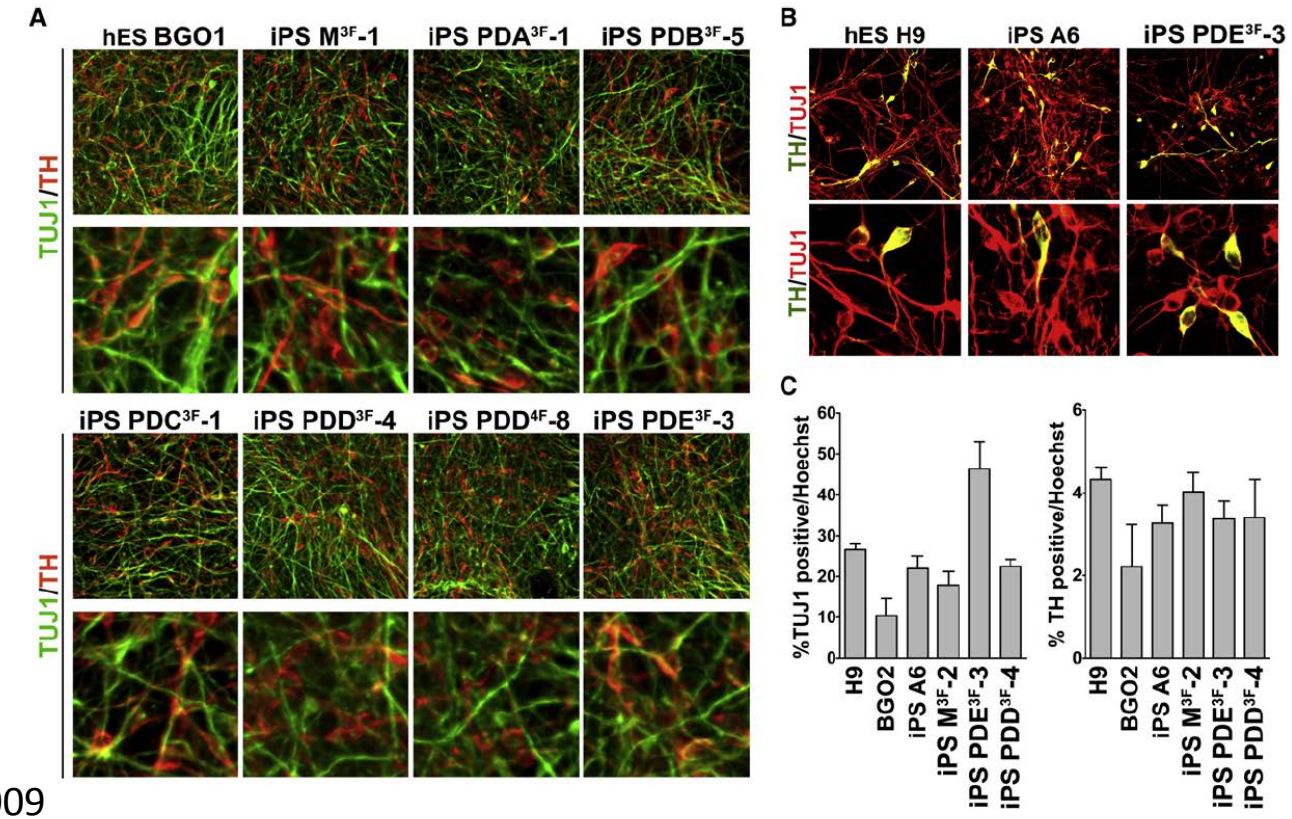
Parkinson's Patient

Cell

Resource

Parkinson's Disease Patient-Derived Induced Pluripotent Stem Cells Free of Viral Reprogramming Factors

Frank Soldner,[1,4] Dirk Hockemeyer,[1,4] Caroline Beard,[1] Qing Gao,[1] George W. Bell,[1] Elizabeth G. Cook,[1] Gunnar Hargus,[3] Alexandra Blak,[3] Oliver Cooper,[3] Maisam Mitalipova,[1] Ole Isacson,[3] and Rudolf Jaenisch[1,2,*]



Soldner et al, 2009

Pharmacological rescue of **mitochondrial deficits** in iPSC-derived neural cells from patients with familial Parkinson's disease

Cooper O, Seo H, Andrabi S, Guardia-Laguarta C, Graziotto J, Sundberg M, McLean JR, Carrillo-Reid L, Xie Z, Osborn T, Hargus G, Deleidi M, Lawson T, Bogetofte H, Perez-Torres E, Clark L, Moskowitz C, Mazzulli J, Chen L, Volpicelli-Daley L, Romero N, Jiang H, Uitti RJ, Huang Z, Opala G, Scarffe LA, Dawson VL, Klein C, Feng J, Ross OA, Trojanowski JQ, Lee VM, Marder K, Surmeier DJ, Wszolek ZK, Przedborski S, Krainc D, Dawson TM, Isacson O. *Sci Transl Med. 2012*

Isogenic Human iPSC Parkinson's Model Shows Nitrosative **Stress-Induced Dysfunction** in MEF2-PGC1α Transcription

Ryan SD, Dolatabadi N, Chan SF, Zhang X, Akhtar MW, Parker J, Soldner F, Sunico CR, Nagar S, Talantova M, Lee B, Lopez K, Nutter A, Shan B, Molokanova E, Zhang Y, Han X, Nakamura T, Masliah E, Yates JR 3rd, Nakanishi N, Andreyev AY, Okamoto S, Jaenisch R, Ambasudhan R, Lipton SA. *Cell. 2013*

Blood cell

Hepatocyte

Skin fibroblast

Diseased patient

Transcription Factors

Plasmids

Small molecules

Oct3/4, Sox2, L-Myc, Lin28

Weeks of factor induction

embryonic-like stem cells
induced pluripotent stem (iPS) cells

Self renwal produces infinite supply of patient-matched cells

Genetic correction through genome editing

Ectoderm: Skin, Brain, Nerve

Mesoderm: Blood, Heart, Kidney, Muscle, Bone

Endoderm: Lung, Stomach, Liver, Pancreas, Intestine

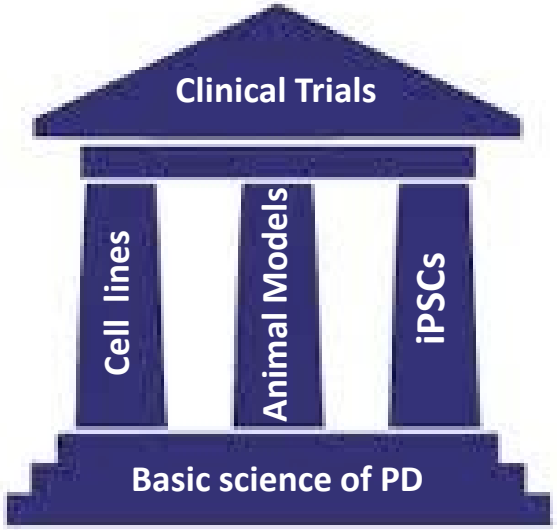
personalized medicine

Modeling PD pathways with iPSCs

- Create iPS lines from PD patients
- Make neurons and other tissues
- Challenge with stressors
- Relate to basic and clinical databases
- Learn about disease mechanisms
- Develop personalized drug therapies

Creating novel therapies for Parkinson's Disease

Three pillars of translational research



Panel Discussion

Panel Discussion -Modeling PD Pathways

Panelists –Mike Lee, Clive Svendsen, Richard Youle, Wade Harper
Moderator –Steve Finkbeiner

3. Support the development of translational resources with greater power to predict efficacy and biomarker outcomes in clinical trials. These resources would include well- characterized, replication sets of non-integrating iPSC lines from sporadic cases and from patients with each major PD-causing gene mutation.

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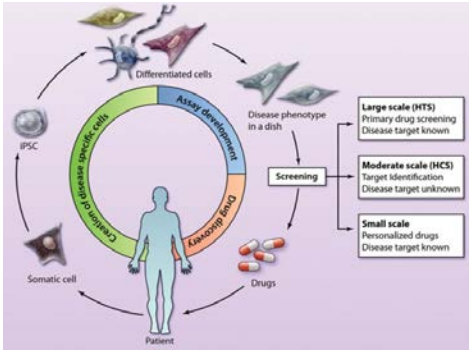
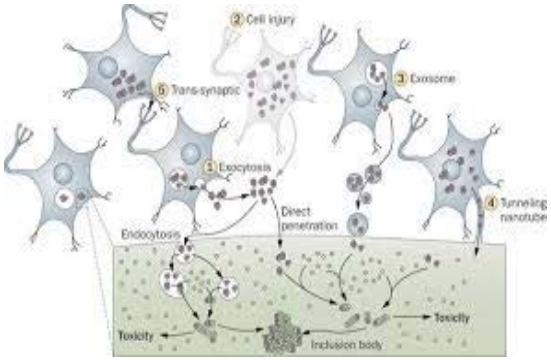




Session 2- Building a Translational Pipeline for Parkinson's Disease Therapeutics

Target Identification and Validation

Richard Youle and Wade Harper

1. To develop treatments that alter the course of disease progression we need a better understanding of the molecular etiology of PD.

2. How do we moving forward in drug discovery with current understanding?

To what extent are there unifying hypotheses among proposed PD etiologies

- #9 Investigate the relationship between α-syn misfolding and mitochondrial function to further understand pathways that intersect in PD
- #8 Develop a thorough understanding of targets and pathways associated with pathogenesis and pathophysiology of PD with emphasis on those that are validated via human genetics and biology
- #10 Develop tools and technologies for measuring pathway architecture and flux in PD and integrate findings from these approaches across 'omics' platforms into a systems level understanding of pathogenesis.

Are there unrelated causes of Parkinsonism?

Alpha Synuclein Misfolding

Genetic evidence

- α-synuclein mutation
- α-synuclein triplication

Pathologic evidence

- Lewy bodies

Mitochondrial Damage Accumulation

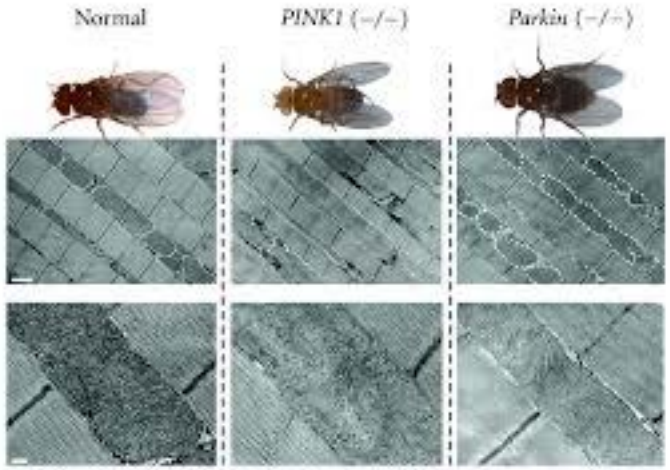
Genetic evidence

- PINK1 kinase
- Parkin ubiquitin ligase
  both autosomal recessive
- mitochondrial DNA polymerase (PolG)

Pathologic evidence

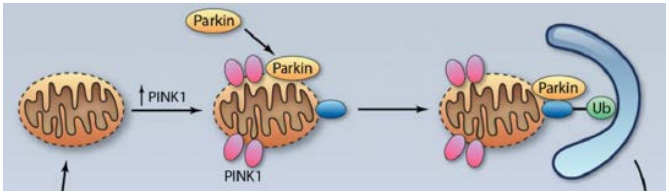
- COX 1 deficiency
- mitochondrial DNA deletions

In fruit flies PINK1 and Parkin are in same pathway - genetically



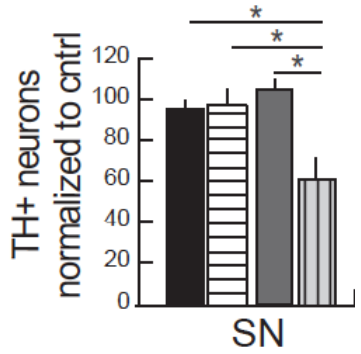
Nature 441:1162, 2006; Nature 441:1157, 2006.

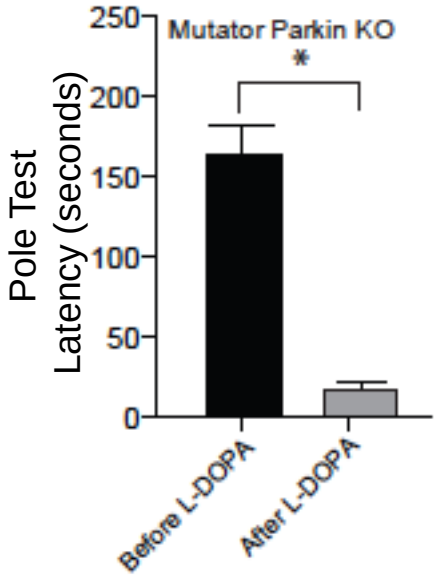
In human IPS neurons PINK1 and Parkin are in the same pathway – biochemically



J Neurosci. 31:5970, 2011

In mouse endogenous Parkin mitigates mitochondrial damage to substantia nigral neurons





Pinto et al., submitted

Is there overlap between α-synuclein and mitochondria?

α-Synuclein Misfolding

Does α-synuclein misfolding affect mitochondrial activity?

Mitochondrial Damage Accumulation

Does mitochondrial impairment affect α-synuclein misfolding?

Unlikely because only a subset of PINK1/Parkin patients display Lewy bodies – unless α-syn is toxic upstream of Lewy body formation

Some PolG PD patients display extensive Lewy body pathology

J. Neurosci 33: 10790, 2013

Recommendation:

We need better assessment/validation in animal models of

- epistasis between α-synuclein toxicity and the PINK1/Parkin pathway
- epistasis between α-synuclein toxicity and genetic causes of mitochondrial damage linked to PD such as PolG mutant mice

To what extent is there overlap in molecular pathways among different forms of PD

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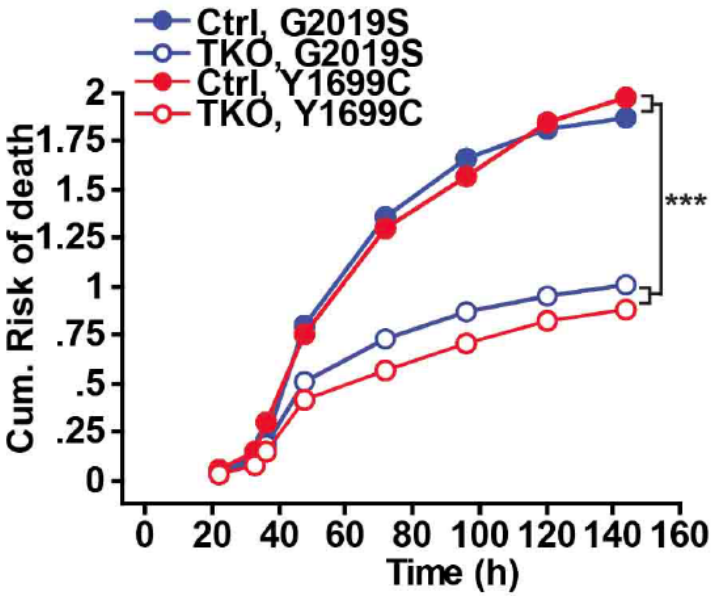
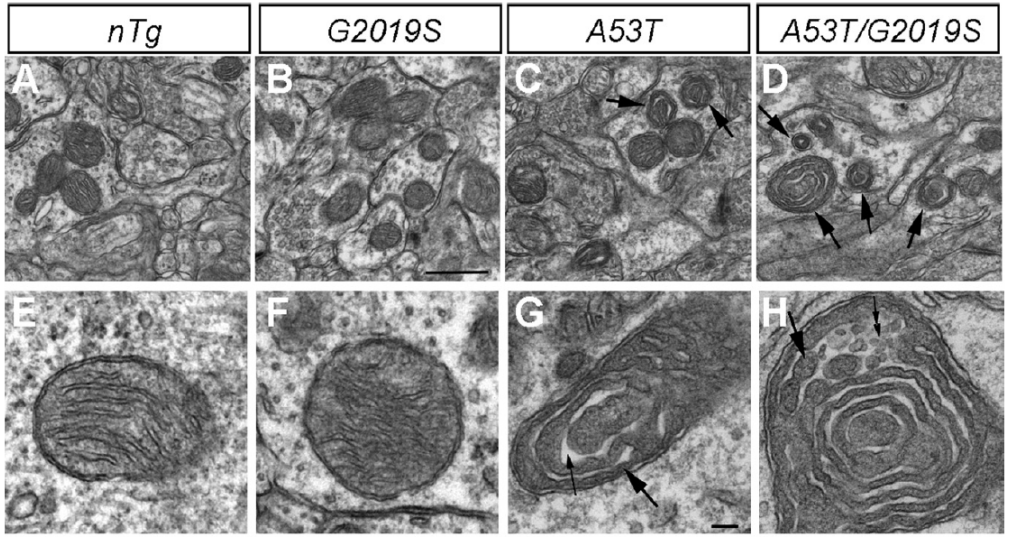
Explore potential interactions among other genes mutated in PD

LRRK2 mutation toxicity is reduced in α &(α,β,γ)-synuclein depleted neurons in culture



Skibinski et al. J. Neurosci. in press

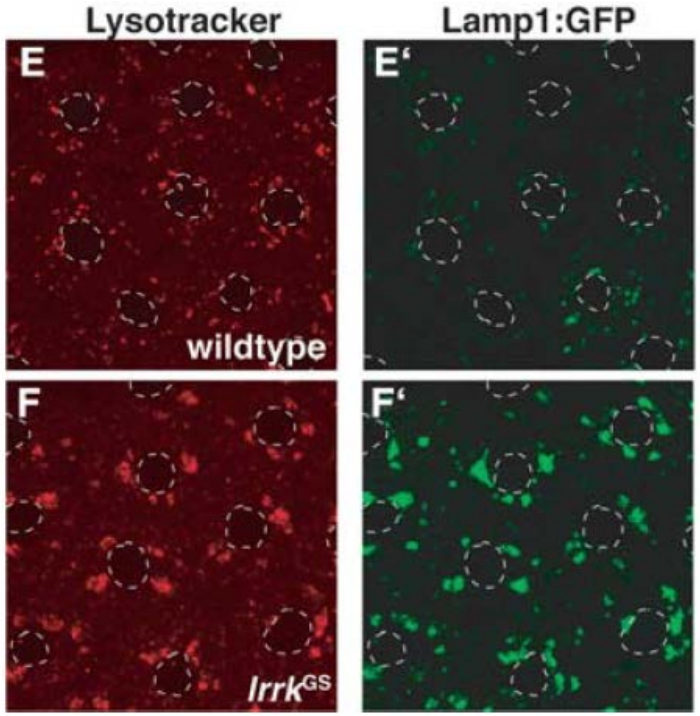
LRRK2 mutation with α-synuclein mutation affects mitochondria *in vivo* in mouse



Lin et al. Neuron 64:807-27, 2009

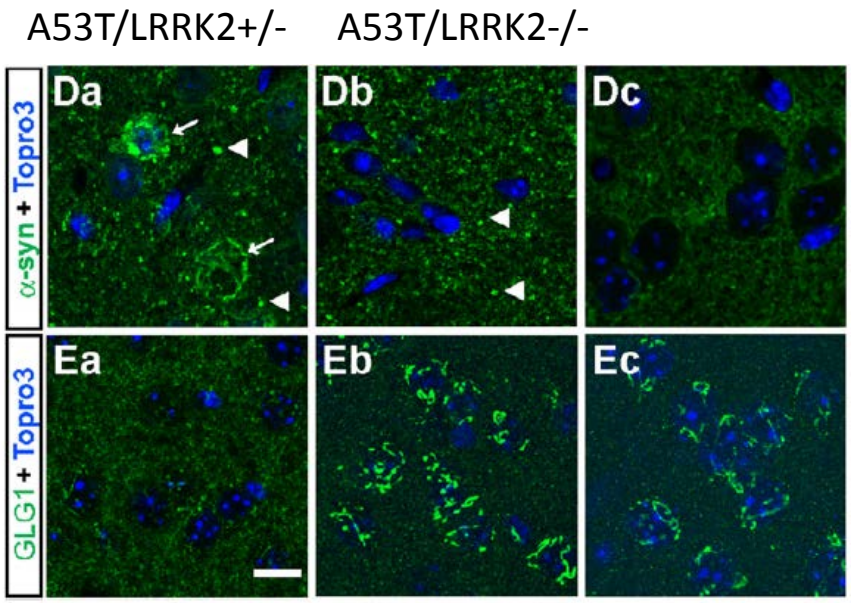
Explore potential interactions among other genes mutated in PD

LRRK2 mutation and the lysosomes *in vivo*



Dodson et al. HMG 21:1350, 2012

LRRK2 mutation with α-syn mutation affects α-syn aggregation and Golgi apparatus *in vivo*



Lin et al. Neuron 64:807-27, 2009

Various intracellular effects of LRRK2 mutation - what is the primary upstream defect?

Explore potential interactions among other genes mutated in PD

Lysosomes
- GBA (glucocerebrosidase)
- ATP13A2

Endocytosis/Retromer complex
- VPS35

Proteosome
- FBXO7

Mitochondrial and quality control defects in a mouse Model of Gaucher disease – links to PD
Osellame LD, et al. Cell Metab. 17:941-53, 2013.

Identification of novel ATP13A2 interactors and their role in a-synuclein misfolding and toxicity
Usenovic M, et al. Hum Mol Genet. 21:3785-94, 2012.

The Parkinson's disease-linked proteins Fbxo7 and Parkin interact to mediate mitophagy.
Burchell VS, et al. Nat Neurosci. 16:1257-65, 2013.

To what extent are there unifying hypotheses among proposed PD disease etiologies

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Target Identification and Validation

Recommendation 10: Develop tools and technologies for measuring pathway architecture and flux in PD and integrate findings from these approaches across OMICS platforms into a systems level understanding of pathogenesis and a blueprint for effective therapeutic intervention.

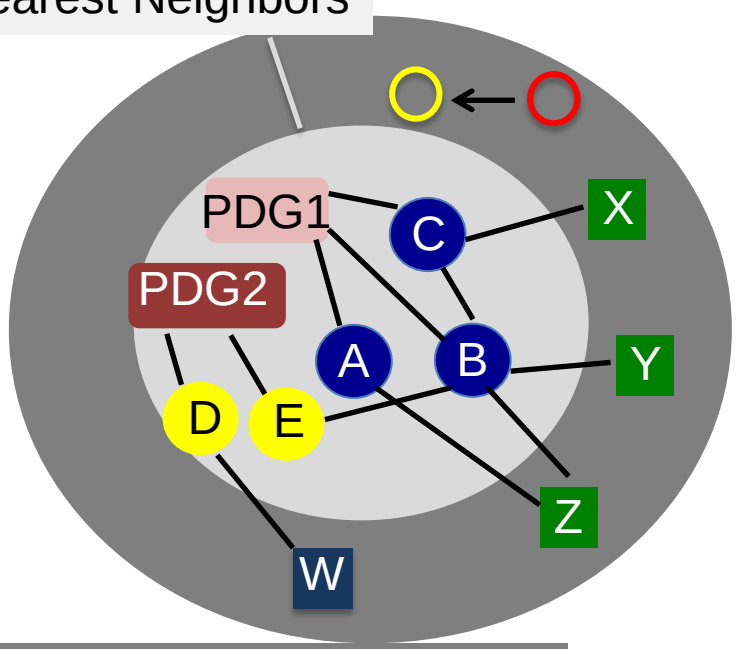
Need: Many of the systems affected in PD and related diseases are dynamic, and involve protein and organelle trafficking. In contrast, the majority of current studies do not examine the flux of molecules through these dynamic systems. To understand the dynamics of state changes at protein and cellular levels , both discovery-based quantification of networks and modifications in different cell states as well as targeted analysis of particular selected proteins and modifications are required.

Approaches:

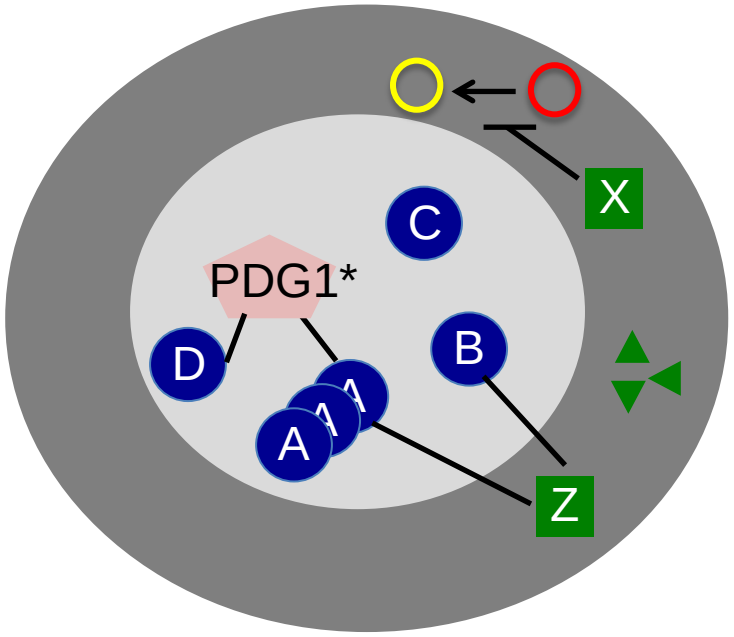
- Determine what networks need to be interrogated (i.e. what networks are most closely related to events that lead to disease), and 2) what technologies are best suited for elucidating changes in state and flux.
- Deploy established and emerging proteomic technologies including AQUA (Absolute Quantification), Tandem Mass Tagging (TMT), and Multiple Reaction Monitoring (MRM) that can allow the dynamics of protein networks to be interrogated on a scale that was not possible even several years ago.
- Develop experimental cell systems, possibly including iPS cells that properly query/model specific genetic defects linked with PD.
- Quantify protein modifications such as phosphorylation, ubiquitylation, and acetylation in both networks as well as at the global level.

A Deeper Understanding of Pathway Architecture and Flux is Needed for Target Identification & Drug Discovery

Primary Interaction Networks
Nearest Neighbors



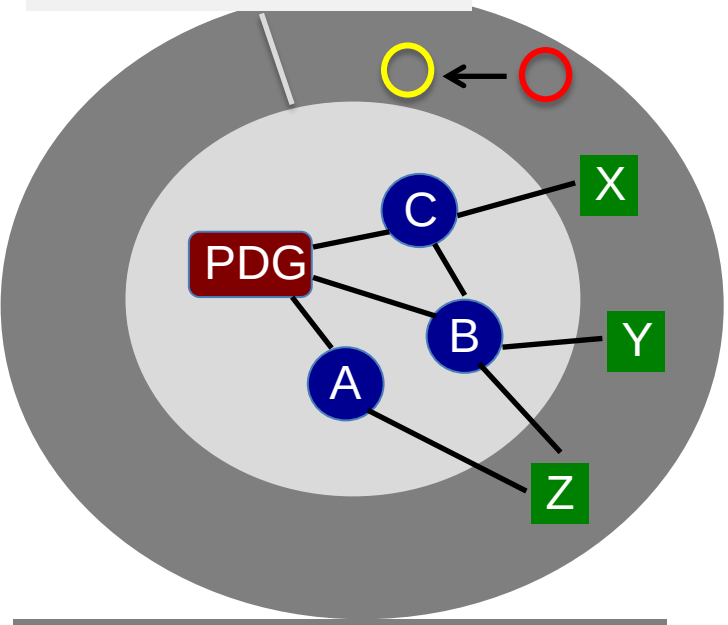
Secondary Interactions
Constellation of metabolome
Global cellular networks



Altered Pathway Architecture
With PD mutant (*)

A Deeper Understanding of Pathway Architecture and Flux is Needed for Target Identification & Drug Discovery

Primary Interaction Networks
Nearest Neighbors



Secondary Interactions
Constellation of metabolome
Global cellular networks

Quantitative Interaction/ Modification Proteomics

- SILAC
- Tandem Mass Tagging (10-plexTMT)
- Reductive Methylation (REDI)
- Absolute Quantification (AQUA) Proteomics

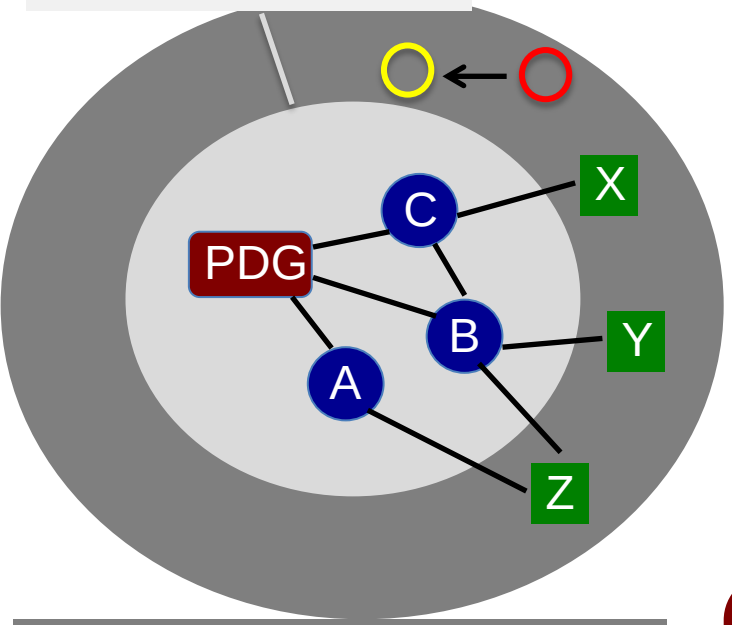
Quantitative Phospho, Ub, etc Capture

+/- PD mutant protein
+/- stimulus
+/- candidate drug

Time
Cell type
etc

A Deeper Understanding of Pathway Architecture and Flux is Needed for Target Identification & Drug Discovery

Primary Interaction Networks
Nearest Neighbors



Secondary Interactions
Constellation of metabolome
Global cellular networks

Quantitative Global Cellular Abundance/ Modification Proteomics

- SILAC
- Tandem Mass Tagging (10-plexTMT)
- Reductive Methylation (REDI)
- Absolute Quantification (AQUA) Proteomics

Quantitative Phospho, Ub, etc Capture

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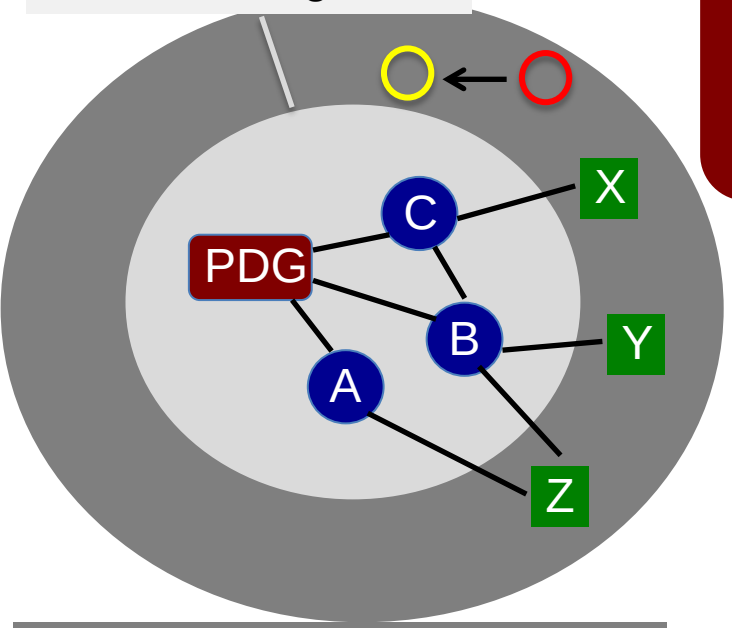
A Deeper Understanding of Pathway Architecture and Flux is Needed for Target Identification & Drug Discovery

Primary Interaction Networks
Nearest Neighbors



Secondary Interactions
Constellation of metabolome
Global cellular networks

Protein/ Organelle/ Metabolic Flux

- Live cell organelle/ protein flux probes (photoactivatable, pH sensitive reporters) — Autophagy, Mitophagy
- Super-resolution Imaging/genomically Encoded Reporters — Bessel Beam Plane Illumination Microscopy
- MRM Proteomics to Examine Targeted Protein Turnover
- Metabolic Tracer Labeling Metabolomics

+/- PD mutant protein
+/- stimulus
+/- candidate drug

Time
Cell type
etc

Overlap of genes identified in large-scale screens

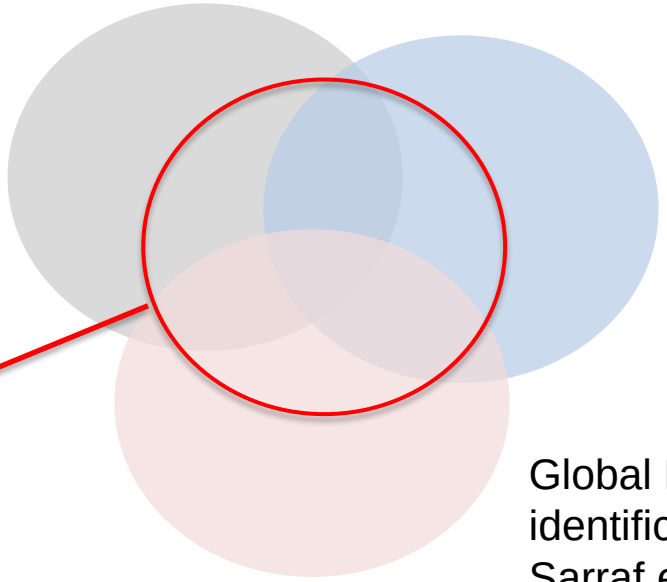
LETTER

doi:10.1038/nature12

High-content genome-wide RNAi screens identify regulators of parkin upstream of mitophagy

Samuel A. Hasson[1,2]*, Lesley A. Kane[1]*, Koji Yamano[1], Chiu-Hui Huang[1], Danielle A. Sliter[1], Eugen Buehler[2], Chunxin Wang[1], Sabrina M. Heman-Ackah[3], Tara Hessa[1], Rajarshi Guha[2], Scott E. Martin[2] & Richard J. Youle[1]

Enhancers and suppressors of PARKIN translocation



pink/*park* large-scale genetic screens in Drosophila (Spyros Artavanis-Tsakonas Lab, unpublished)

Overlapping genes and Pathways may represent Candidate targets for drug discovery

Global PARKIN substrate identification by proteomics Sarraf et al., Nature 2013

Technology Development in Network/Trafficking Tools

- Invest in genomically encoded flux and pathway reporters and related tools for engineering iPS cells, neurons, and animals with reporters for relevant organelles and pathways to enable drug/mechanism discovery

- Encourage the use of physiologically and genetically defined cell systems that provide the appropriate context of candidate target/drug discovery

- Develop standards for the field for pathway architecture, dynamics, and flux determination/reporting

- Invest in super resolution technology development and its application to trafficking in neurons

- Provide a foundation for the development of metabolomic dynamics measurements and its application to relevant patient samples for biomarker discovery

To what extent are there unifying hypotheses among proposed PD disease etiologies

How can pathway analysis and model systems be employed to accelerate target and drug discovery

- #8 Develop a thorough understanding of targets and pathways associated with pathogenesis and pathophysiology of PD with emphasis on those that are validated via human genetics and biology
- #10 Develop tools and technologies for measuring pathway architecture and flux in PD and integrate findings from these approaches across 'omics' platforms into a systems level understanding of pathogenesis

Approaches for therapeutic intervention rely on understanding genetics and mechanism

Dominant Mutations	Recessive Mutations
Remove toxic protein	Activate mutant alleles
Bypass toxicity	Activate downstream defect

Eliminating the effects of dominant mutants (α-SYN, LRRK2)

Remove toxic protein → Antisense oligonucleotides (ASOs)

↓

General elevation of autophagy and lysosomal function by small molecules to facilitate misfolded protein/ aggregate removal

Rapamycin – protects against neuronal cell death in vitro and in vivo

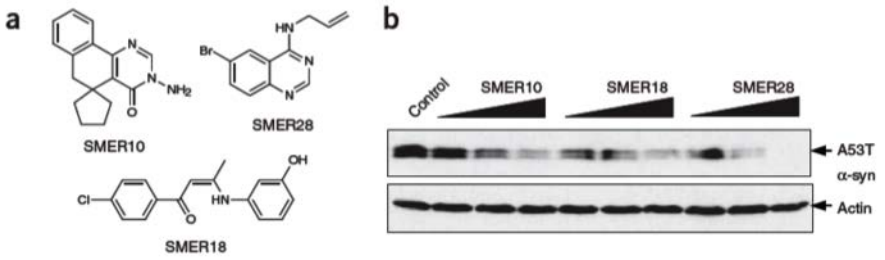
Beclin overexpression – protects against activates autophagy and ameliorates α-SYN toxicity

SMERs – mTOR-independent autophagy activators promote α-SYN degradation

Trehalose – "chemical chaperone" and autophagy enhancer promote α-SYN degradation

In Phase I for reversal of SMN splicing defects in SMA patients

In preclinincal development for other dominant mutations in e.g. ALS (Lagier-Tourenne et al PNAS 2013)

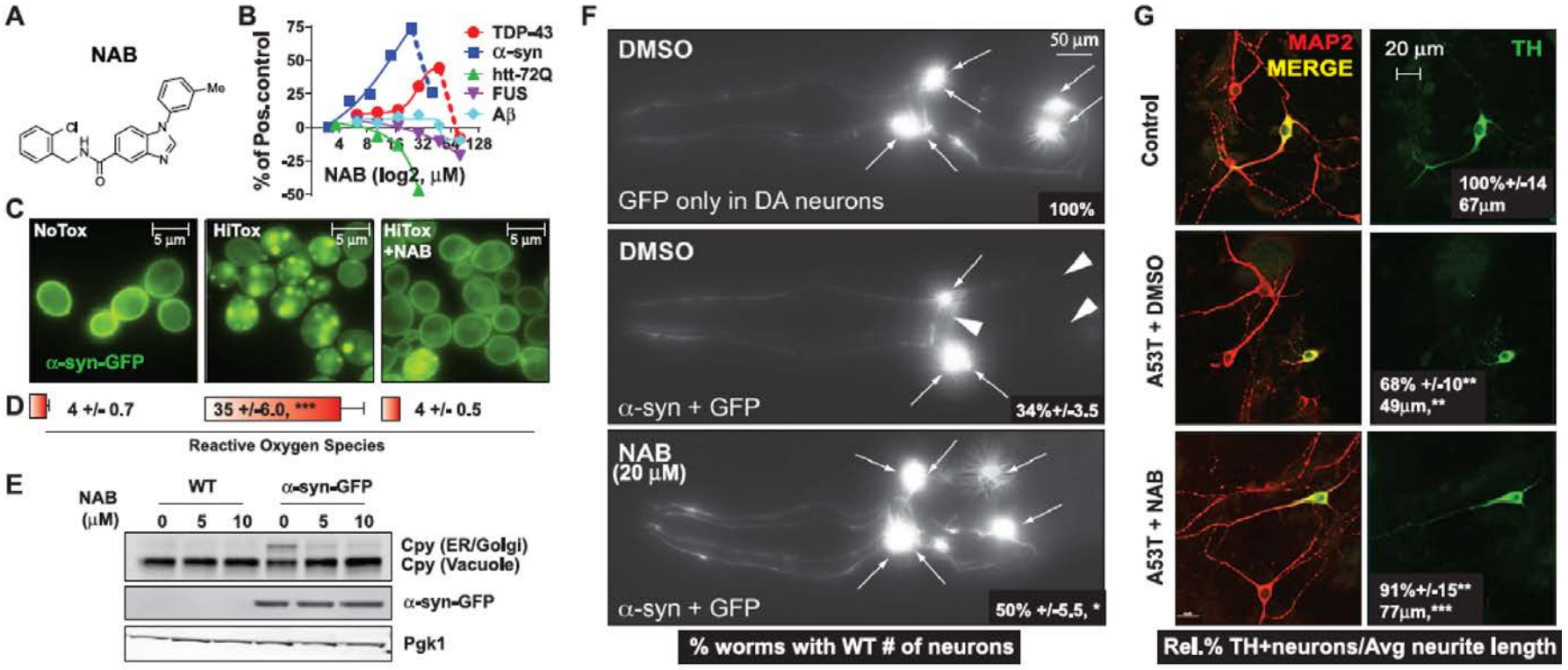


Show additive effects with Rapamycin

Sarkar et al Nat Chem Biol, 2007

Yeast model systems for small-molecule discovery in PD

Chemical and genetic screens for reducing α-SYN toxicity (Lindquist Lab)



Tardiff et al. Science 2013

Systematic network analysis in yeast

Phenotypes identified in yeast (including NO stress and ERAD dysfunction are also present in a-SYN A53T iPS cells and can be reversed by NAB2 and also be expression of NEDD4 (Chung et al, Science 2013; Lindguist Lab)

Patient-derived iPS cells may be a tool to examine phenotypes identified in model systems and then test whether altering those phenotypes increases neuronal viability, etc

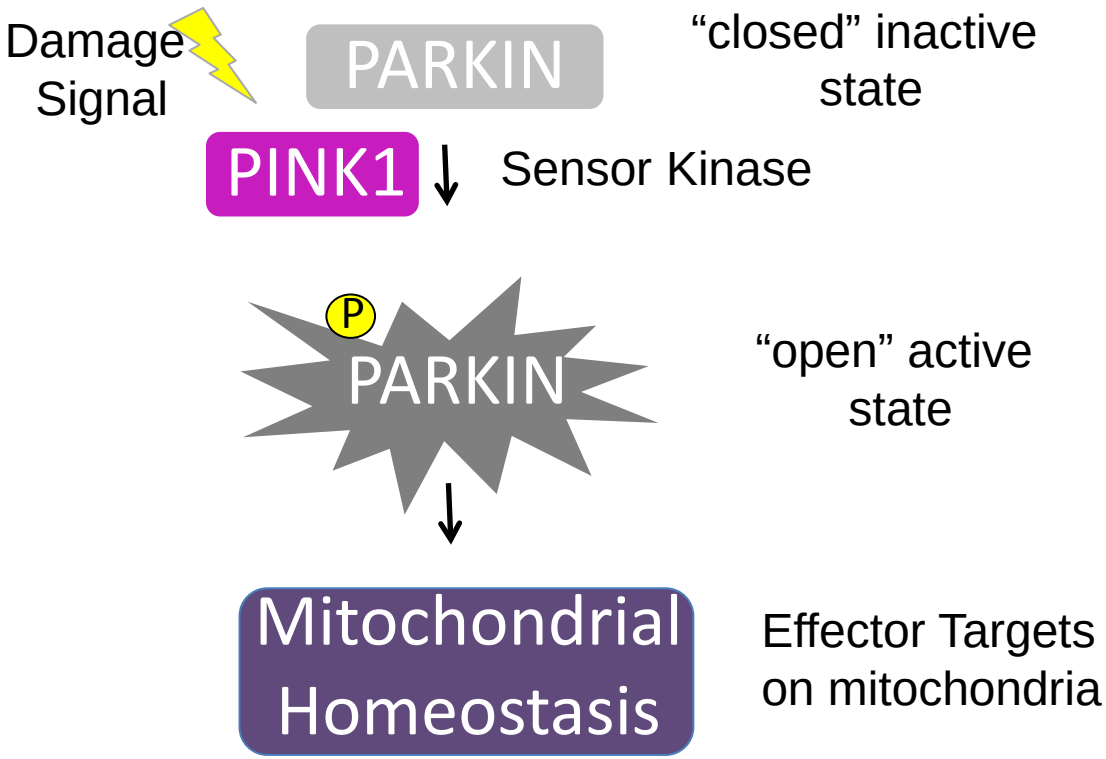
Tardiff et al. Science 2013

Approaches for therapeutic intervention rely on understanding genetics and mechanism

Recessive Mutations

Activate mutant alleles

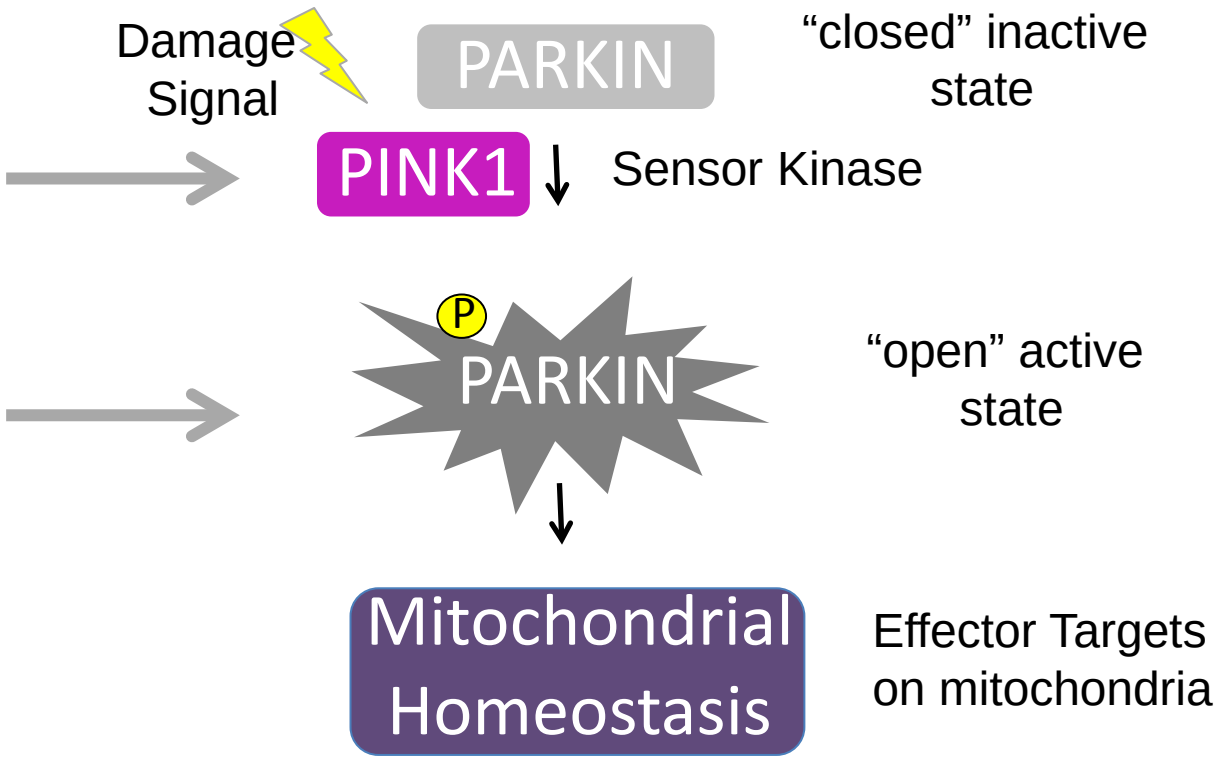
Activate downstream defect



Approaches for therapeutic intervention rely on understanding genetics and mechanism

Molecules that activate Mutant alleles of PINK1 (Shokat Lab, Cell 2013)

Hypothetically, it may be possible to find small molecular activators of mutant PARKIN alleles (could be challenging based on structure of inactive PARKIN)



Going Forward with Target-Drug Discovery

Alternative model systems for drug discovery – for example, building alternative disease models in yeast and Drosophila

Large-scale genetic suppressor and enhancer studies to identify upstream and downstream druggable targets – already substantial progress being made in Drosophila in the context of PINK and PARK mutations

Can analogous approaches be leverages in iPS cells/neurons at the scale necessary for target/drug discovery???

Panel Discussion

Target Identification and Validation

Panelists - Richard Youle, Dean Jones, Rudi Balling, John Hardy, Dimitri Krainc
Moderator - Wade Harper

- Develop a thorough understanding of targets and pathways associated with pathogenesis and pathophysiology of Parkinson's disease with emphasis on those that are validated via human genetics and biology.

- Investigate the relationship between alpha synuclein misfolding and mitochondrial function to further understand pathways that intersect in Parkinson's disease.

- Develop tools and technologies for measuring pathway architecture and flux in PD and integrate findings from these approaches across OMICS platforms into a systems level understanding of pathogenesis and a blueprint for effective therapeutic intervention.

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Recommendation 8: Develop a thorough understanding of targets and pathways associated with pathogenesis and pathophysiology of Parkinson's disease with emphasis on those that are validated via human genetics and biology.

Need: There is a need to agree on the most compelling and key pathways that are emerging in Parkinson's disease and have a strong foundation in human genetics or human biology, and to focus rigorous efforts to determine the most promising of these for future therapeutic development. At the same time there is a need to identify new promising targets that have not yet been identified.

Approaches:

- Confirm the validity of targets and pathways using human derived information such as genetics, tissue and fluid omics approaches.
- Develop a well curated biobank including brain tissue, CSF, blood, plasma, DNA, skin etc.
- Determine whether there are convergent pathway(s) between dominant and recessive Parkinson's disease and whether this is contributing to sporadic PD
- Genetic screens in *C. elegans* or Drosophila for enhancers and suppressors of genes already linked to PD may reveal new genetic steps in the known PD pathways and identify new drug targets. Cell based RNAi screens assessing wild type or mutant alpha synuclein expression, aggregation or autophagic engulfment might also be explored. Chemical genomic strategies stemming from cell based phenotypic drug screens could yield new drug targets following identification of the chemical targets.
- Investigate the role of organelle trafficking deficits in PD pathogenesis.

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Recommendation 9: Investigate the relationship between alpha synuclein misfolding and mitochondrial function to further understand pathways that intersect in Parkinson's disease.

Need: There is strong evidence for both alpha synuclein misfolding and mitochondrial dysfunction in PD. One key question for translational drug development is whether these are unrelated causes of PD or if these two processes intersect to cause neuron death. Autosomal recessive Parkinson's Disease (ARPD), such as those caused by parkin mutations, are associated with selective loss of DA neurons, lack of non-motor deficits, lack of alpha-synuclein pathology, and slow progression (some lasting several decades). These clinical features are very different from idiopathic PD.

Approaches:

- Determine what is needed for disease modifying therapy for ARPD patients.
- Determine the best predictive models for preclinical evaluation.
- Explore phenotypically relevant mouse models of alpha synuclein mutation crossed into Parkin -/-, Pink1 -/-, or mitochondrial POLG mutant (Mutator) backgrounds or other mitochondrial stressed mice to determine if there is synergy between alpha synuclein aggregation and mitochondrial stress in causing dopaminergic neuron loss. One could also consider iPSC models from patients to assess crossover between the two pathways *in vitro*.

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Recommendation 10: Develop tools and technologies for measuring pathway architecture and flux in PD and integrate findings from these approaches across OMICS platforms into a systems level understanding of pathogenesis and a blueprint for effective therapeutic intervention.

Need: Many of the systems affected in PD and related diseases are dynamic, and involve protein and organelle trafficking. In contrast, the majority of current studies do not examine the flux of molecules through these dynamic systems. To understand the dynamics of state changes at protein and cellular levels , both discovery-based quantification of networks and modifications in different cell states as well as targeted analysis of particular selected proteins and modifications are required.

Approaches:

- Determine what networks need to be interrogated (i.e. what networks are most closely related to events that lead to disease), and 2) what technologies are best suited for elucidating changes in state and flux.
- Deploy established and emerging proteomic technologies including AQUA (Absolute Quantification), Tandem Mass Tagging (TMT), and Multiple Reaction Monitoring (MRM) that can allow the dynamics of protein networks to be interrogated on a scale that was not possible even several years ago.
- Develop experimental cell systems, possibly including iPS cells that properly query/model specific genetic defects linked with PD.
- Quantify protein modifications such as phosphorylation, ubiquitylation, and acetylation in both networks as well as at the global level.

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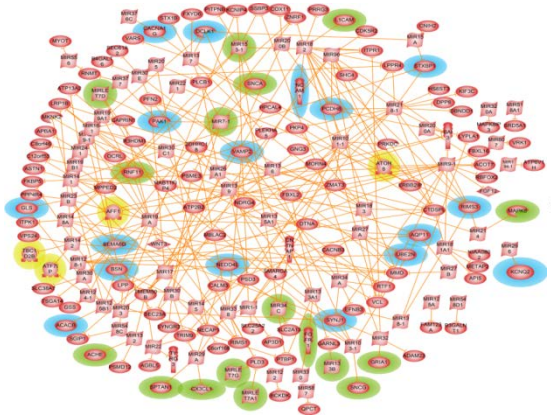
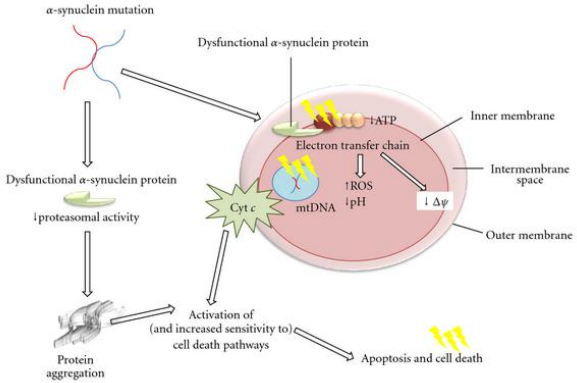
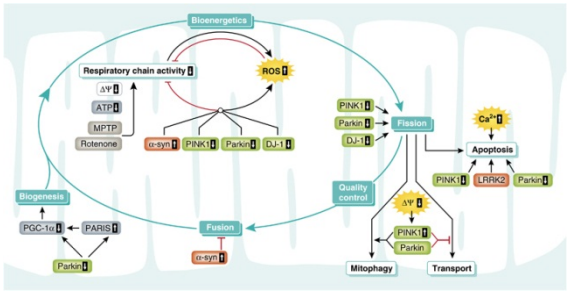
- Develop tools and technologies for measuring pathway architecture and flux in PD and integrate findings from these approaches across OMICS platforms into a systems level understanding of pathogenesis and a blueprint for effective therapeutic intervention.







Panel Discussion

Ranking	Translational Working Group Recommendations
1	Develop patient stratification strategies and support technologies that define disease signatures
2	Develop novel and specific α-synuclein PET imaging agents and assays for α-synuclein burden
3	Develop translational resources that lead to drugs with improved predictive efficacy and outcomes in clinical trials
4	Support the development of an integrated PD database inclusive of data from human genetic, biomarker and clinical studies
5	Develop consensus guidelines and standards for the use of existing PD pathway models
6	Develop and apply intermediate markers of drug efficacy in early Parkinson's disease translational studies
7	Define required attributes of targets emerging from basic science for advancement in translational studies
8	Develop a thorough understanding of targets and pathways validated through human genetic and biology studies
9	Investigate the relationship between α-synuclein misfolding and mitochondrial function.
10	Develop tools and technologies for measuring pathway architecture and flux in PD