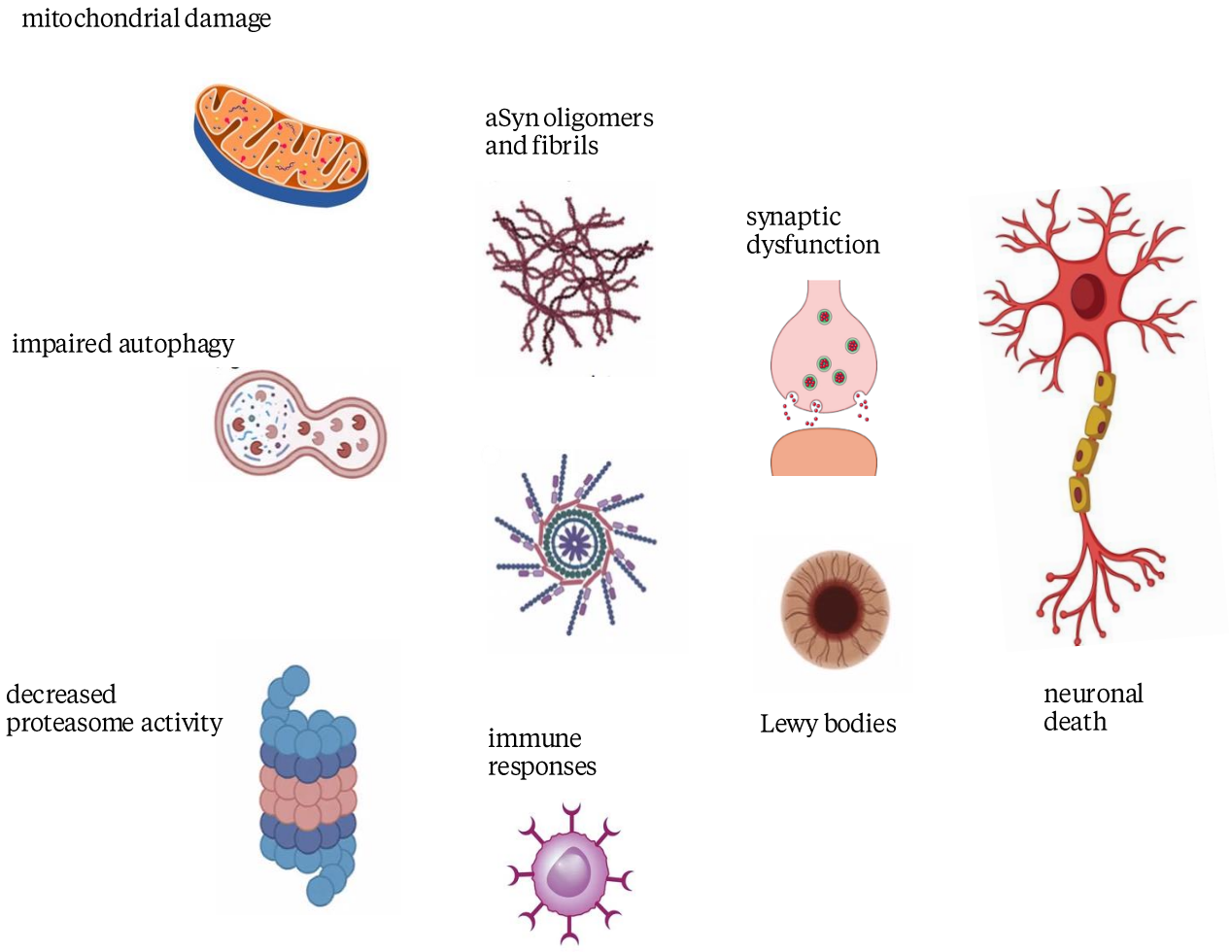
Aligning in vivo models of Parkinson's disease with biological classifications

Patrick Howson, Chief Innovation Officer

Parkinson's disease

- A progressive neurodegenerative disorder
- Classically considered a movement disorder but with many non-motor components
- Pathologically associated with dopaminergic degeneration and alpha-synuclein aggregation in Lewy bodies
- Parkinson's disease is the fastest growing neurodegenerative disease
- >7M people living with Parkinson's worldwide
- $52B annual economic burden in US
- Symptomatic treatments exist, but there are no disease modifying therapies
- It is a heterogenous disease – different individuals present with different symptoms and progress at different rates



Modified from Morris et al (2024) Lancet 403:293

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Commonly used animal models

	Neurotoxin	α–Synuclein based	Transgenic
EXAMPLES	6-OHDA, MPTP, rotenone, paraquat	AAV-aSyn aSyn PFF	aSyn based (M83, G2-3) Non-aSyn (LRRK2, Parkin, Pink1)
WHAT THEY MODEL WELL	Loss of DA neurons Motor deficits Motor complications	Synucleinopathy Neuronal dysfunction Neuronal loss (variable)	Age dependent effects Specific genetic causes of PD
STRENGTHS	Low cost Reproducible Rapid Well characterised	Pathologically relevant Progressive (still rapid)	Progression over lifetime Relevant genetic mechanism
LIMITATIONS	Limited progression Pot. for high mortality Biosafety considerations Batch-to-batch variability Surgical delivery (some)	Neuronal loss can be limited Limited spread (AAV) Variability between groups (PFF) Less well characterised Surgical delivery	High cost Limited neuronal loss death Long studies (drug develop.)
TYPICAL USE	Symptomatic therapies Cell replacement Neuroprotection Environmental factors	Disease modifying therapies Immunotherapies Aggregation inhibitors aSyn spread/ pathology	Studying aSyn biology Studying the development of motor/ non-motor symptoms Mechanistic pathways

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Predictive validity of models

Anti–parkinsonian benefits

COMPOUND CLASS	RODENT	NHP	PHASE II
D2 dopamine agonists	Yes	Yes	Yes
A2A adenosine antagonists	Yes	Yes	Yes
COMT inhibitors	Yes	Yes	Yes
MAO-B inhibitors	Yes	Yes	Yes
DAT/ NET/ SERT inhibitors	Yes	Yes	Mild/ No
mGlu 4 PAMs	Yes	Yes	N/A
delta opioid agonists	Yes	Yes	N/A
NR2B NMDA antagonist	Yes	Yes	No
D1 positive allosteric modulators	Yes	Yes	Yes

Anti–dyskinetic benefits

COMPOUND CLASS	RODENT	NHP	PHASE II
NMDA receptor antagonists (AMT)	Yes	Yes	Yes
NR2B NMDA antagonists	Yes	No	No
mGlu5 NAMs	Yes	Yes	Yes
AMPA antagonists	Yes	Yes	No
alpha adrenergic antagonists	Yes	Yes	Yes
SV2A	Yes	Yes	Yes
5-HT1A agonists	Yes	Yes	Yes
5-HT1B/D antagonists	Yes	Yes	Yes
5-HT2A antagonists/inverse agonists	Yes	Yes	Yes
CB1 agonists	Yes	Yes	Yes
D1 positive allosteric modulators	Yes	Yes	N/A

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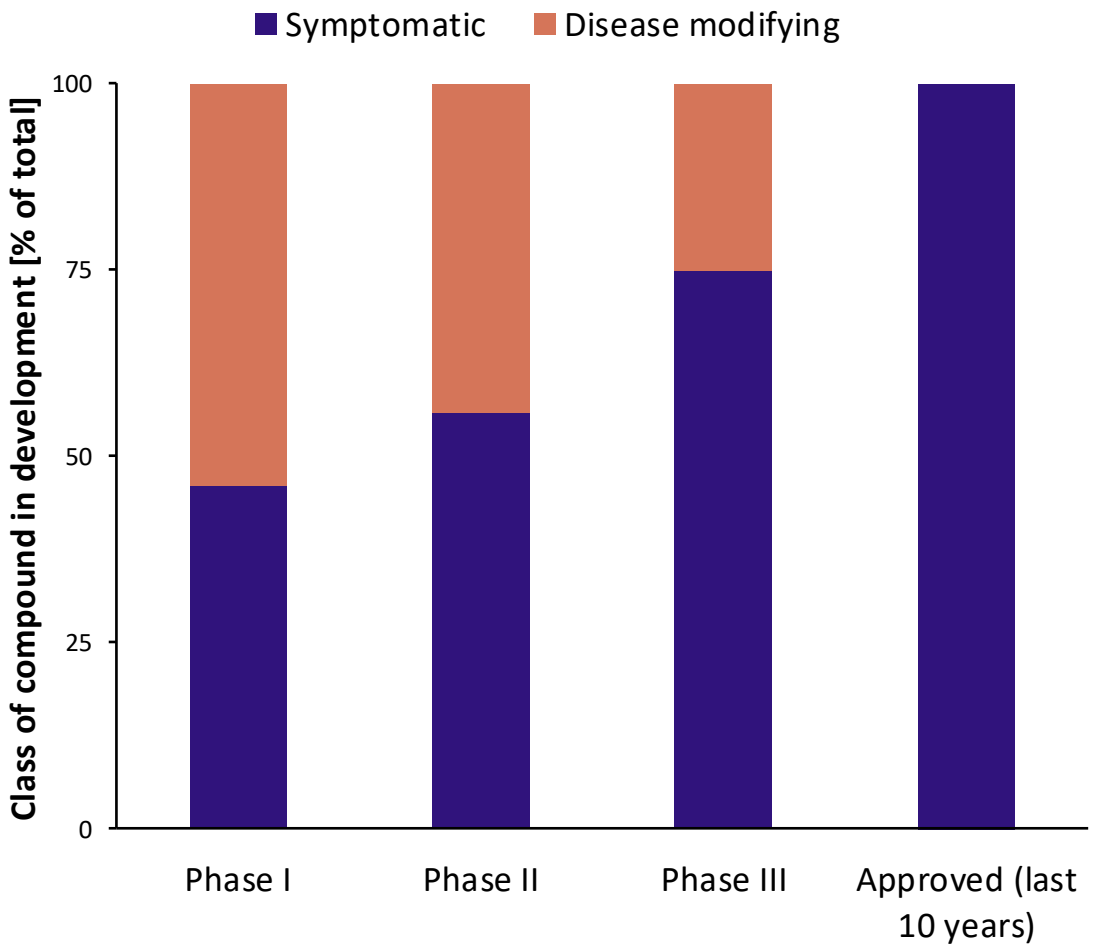
Attrition in PD Drug Development



The increased difficulty in developing disease modifying therapeutics is shown by increased attrition from Phase I → approval, so that, in 2024:

- Approximately equal numbers of symptomatic and disease modifying therapies in Phase I development
- Greater attrition rate for disease modifying therapies during clinical development
- Only symptomatic therapies have been approved for PD

Suggests that there is not a paucity of drug candidates

Reasons for increased attrition?

- Heterogeneity in target population
- Lack of translatability from preclinical to Phase II?
- Insufficient quality of preclinical studies
- Difficult or unvalidated therapeutic targets?
- Issues with disease modifying drug candidates?
- Length and cost of disease modifying therapy development

What can we improve to reduce the attrition rate of disease modifying therapies?

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Models for Symptomatic & Disease Modifying Therapies

Symptomatic therapies

Reduce severity of symptoms once developed

Symptoms driven by a **state** — the process leading to that state ***may*** be unimportant

- L-DOPA works in PD and animal models despite different processes reducing striatal dopamine

However, the model state must accurately reflect the disease state

- PD and MSA both have reduced striatal dopamine, L-DOPA only effective in people with PD (MSA state ≠ PD state)

Neurotoxin models recapitulated the motor dysfunction state and have excellent predictive validity for symptomatic therapies treating PD and motor complications.

Not currently enough evidence to know whether neurotoxin models will have predictive validity for symptomatic therapies treating non-motor complications

Disease modifying therapies

Alter an ongoing process, which may only be occurring in sub-populations and only at certain disease stages

Disease modifying therapies attempt to alter a disease **process** so that the emergence, or worsening, of a symptom is delayed

The process being modulated must be similar in the animal model and in PD

- Selection of clinical cohort with relevant pathological process. Selection based on clinical criteria may not be sufficient.
- Need to define cohorts based around underlying pathology, not clinical symptoms (biological classification of PD)

Incomplete knowledge of how disease processes differ across PD and how the processes may evolve over time within an individual.

There are knowledge gaps as to which animal models best replicate specific disease processes

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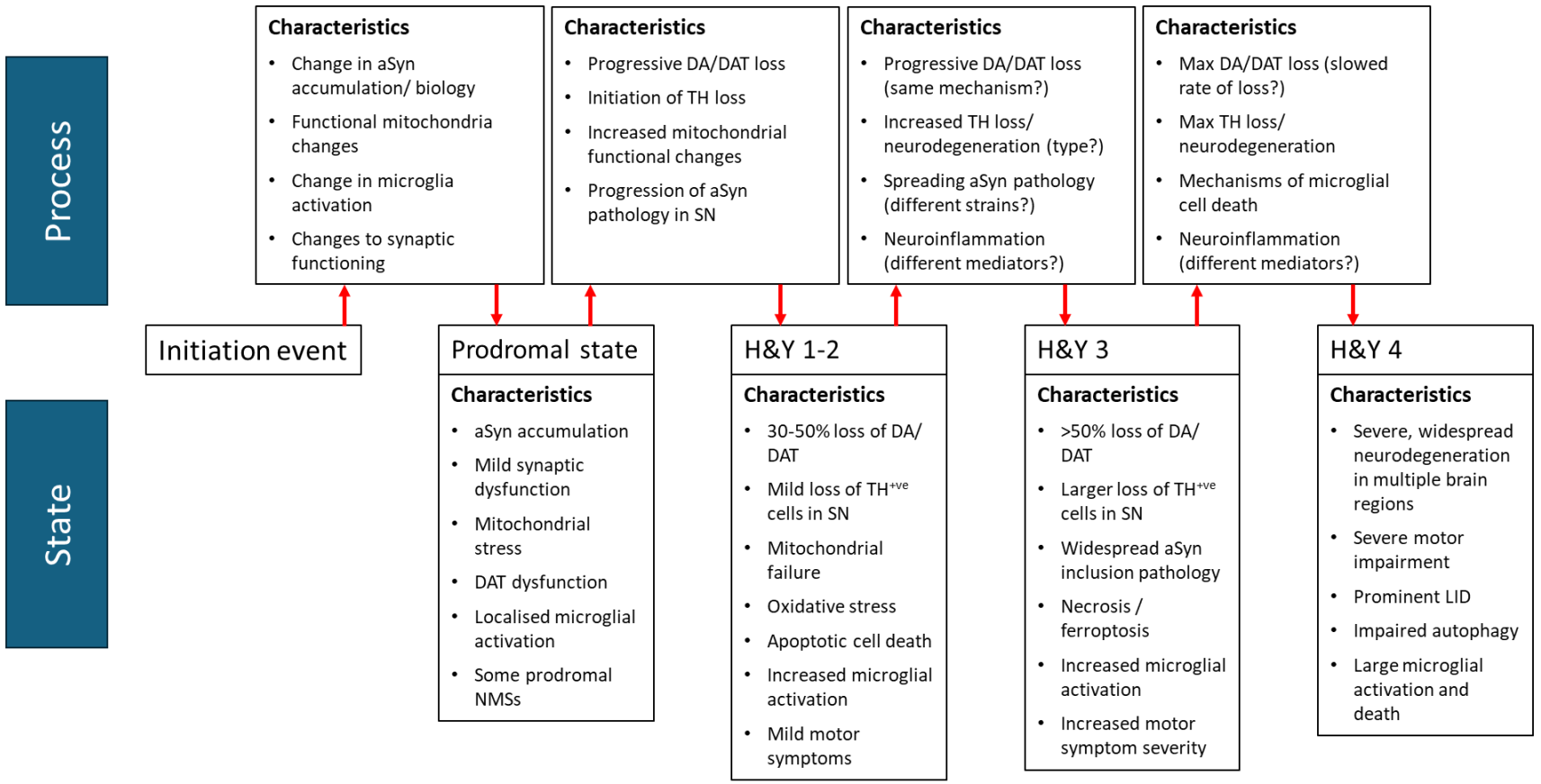
Symptomatic vs. Disease modifying



To develop a disease modifying therapy you need to modulate the ***processes*** that cause disease progression

To develop a symptomatic therapy, you need to use an animal model that replicates the ***state*** of the intended population

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Improvement 1: Heterogeneity in PD

- **Clinical heterogeneity — different symptoms**
  - Motor differences – e.g., tremor dominant
  - Non-motor differences – present with a range of NMSs
- **Pathological heterogeneity — different brain pathology**
  - Location and spread of Lewy pathology differs between individuals
- **Genetic heterogeneity**
  - Different monogenic forms, genetic risk factors
- **Progression heterogeneity — variable disease course**
  - Fast vs. slow progressors
  - Development of symptoms (e.g., cognition) occur at very different times
- **Therapeutic heterogeneity — differing response to treatment**
  - Benefits and side effects differ between individuals

Key Insights

- Heterogeneity is a likely reason for increased attrition of disease modifying therapies (likely less heterogeneity in states than in processes)
- A one-size-fits-all approach for disease modifying therapies is inadequate
- Recent biological classifications of PD offer a way of grouping cohorts of people with PD based around pathology, thus has the potential to reduce heterogeneity
- Need to ensure that animal models replicate the disease processes occurring in these cohorts

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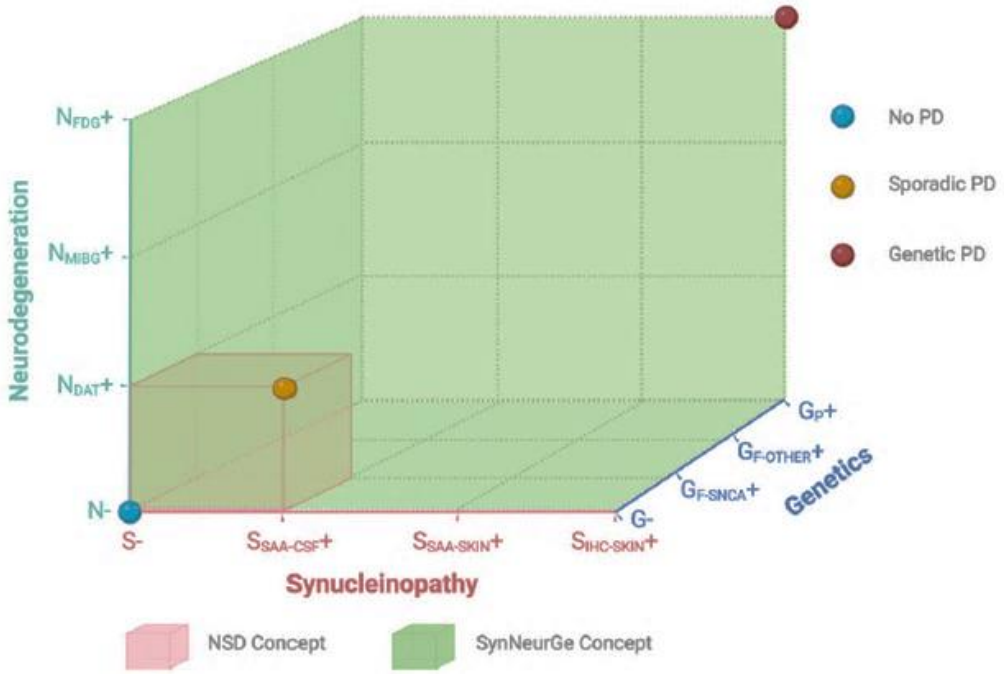
Improvement 1: Biological classifications in PD

Hoglinger and Lang, 2024



Recently, two biology-based criteria in PD were proposed; Synucleinopathy-Neurodegeneration-Genetics (**SynNeurGe**) and Neuronal α-Synuclein Disease Integrated Staging System (**NSD-ISS**)

- Both are significantly different from traditional clinical definitions, emphasizing the importance of objective biological markers.
- NSD-ISS is a more restricted definition of PD that largely fits as a sub-entity within the broader SynNeurGe classification, which captures all people with PD.

- Both classifications use synucleinopathy, neurodegeneration, and genetics as biological anchors
- Both allow for describing a more homogenous cohorts
- Future developments may include additional anchors and/or refinement of current anchors
- They should aid the identification of cohorts that have the same process that is being therapeutically targeted

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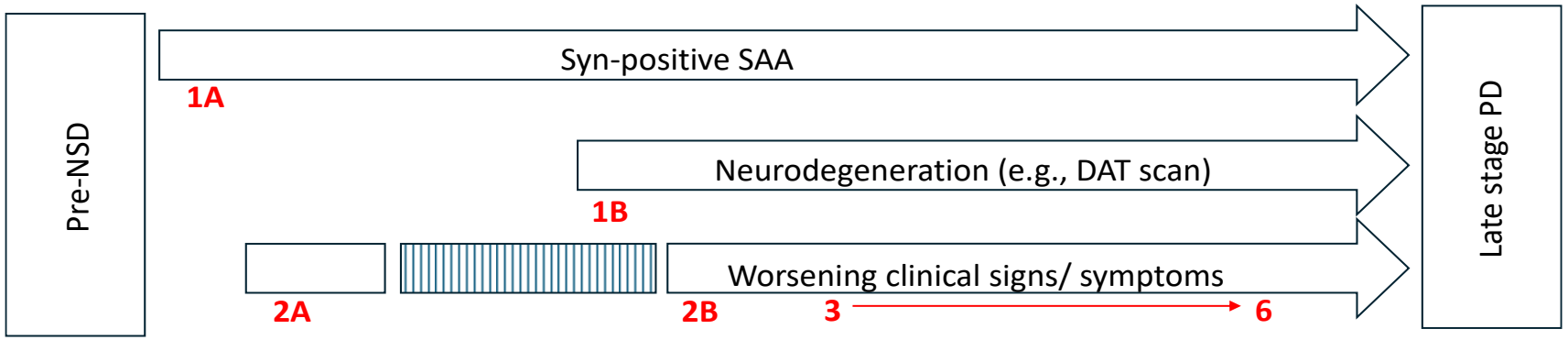
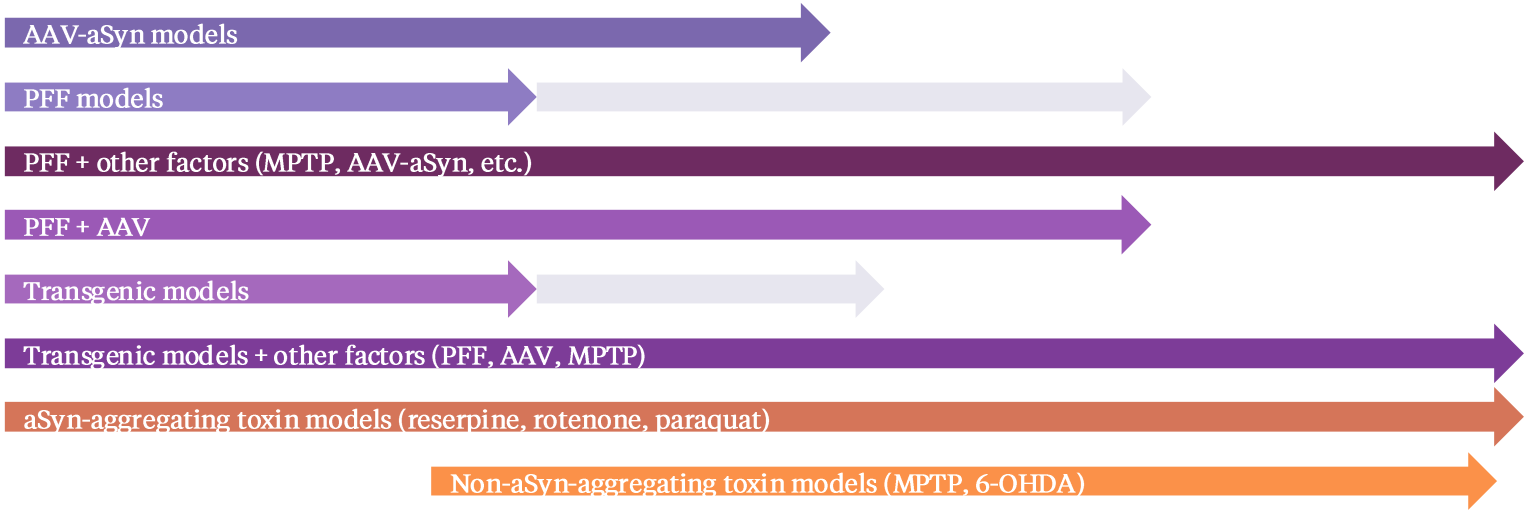
Improvement 2: Aligning biological classifications and animal models



Simuni et al., stages

Models



Useful as a starting point but this is aligning the models with **states** not **processes**

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Improvement 2: States and processes modelled well

States / Processes	Models	What is modelled well
STATES		
Nigrostriatal DA degeneration	6-OHDA, MPTP, AAV-aSyn	Selective vulnerability of SNpc DA neurons. Striatal DA depletion. Motor deficits responsive to L-DOPA
Motor circuit dysfunction	6-OHDA, MPTP, AAV-aSyn	Bradykinesia, rigidity, akinesia. Abnormal basal ganglia output. L-DOPA-induced dyskinesias
Non-motor circuit dysfunction	6-OHDA, MPTP, AAV-aSyn	Predictive validity for NMSs less rigorously tested than for motor symptoms
PROCESSES		
aSyn aggregation & misfolding	aSyn transgenic, PFF, AAV-aSyn	pSer129 inclusion formation. Templating
Spread of pathology	aSyn PFF	Cell-to-cell transmission. Connectivity-dependent spread
Neuroinflammation	MPTP, rotenone, PFF, AAV-aSyn	Microglial activation. NLRP3 signaling. Cytokine release. Adaptive immune system
Mitochondrial dysfunction	MPTP, rotenone, PINK1, Parkin	Complex I inhibition. ROS generation. Mitophagy defects
Proteostasis failure	GBA1, LRRK2, VPS35, CTSB models	Impaired lysosomal activity. Autophagic flux defects. aSyn accumulation

Many states and processes not modelled well: NMSs, slow prodromal evolution, development of Lewy bodies, non-cell-autonomous degeneration

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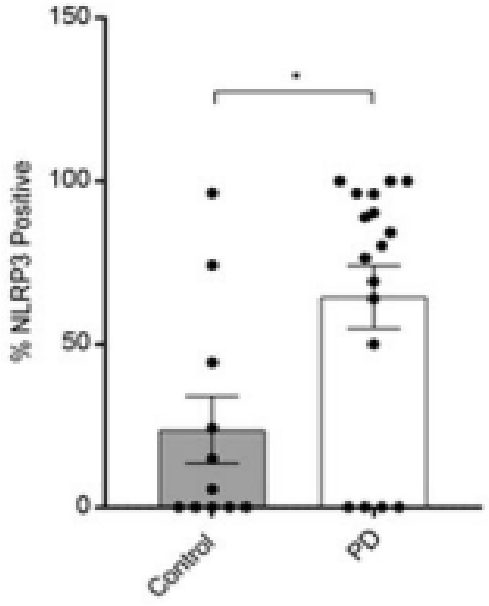
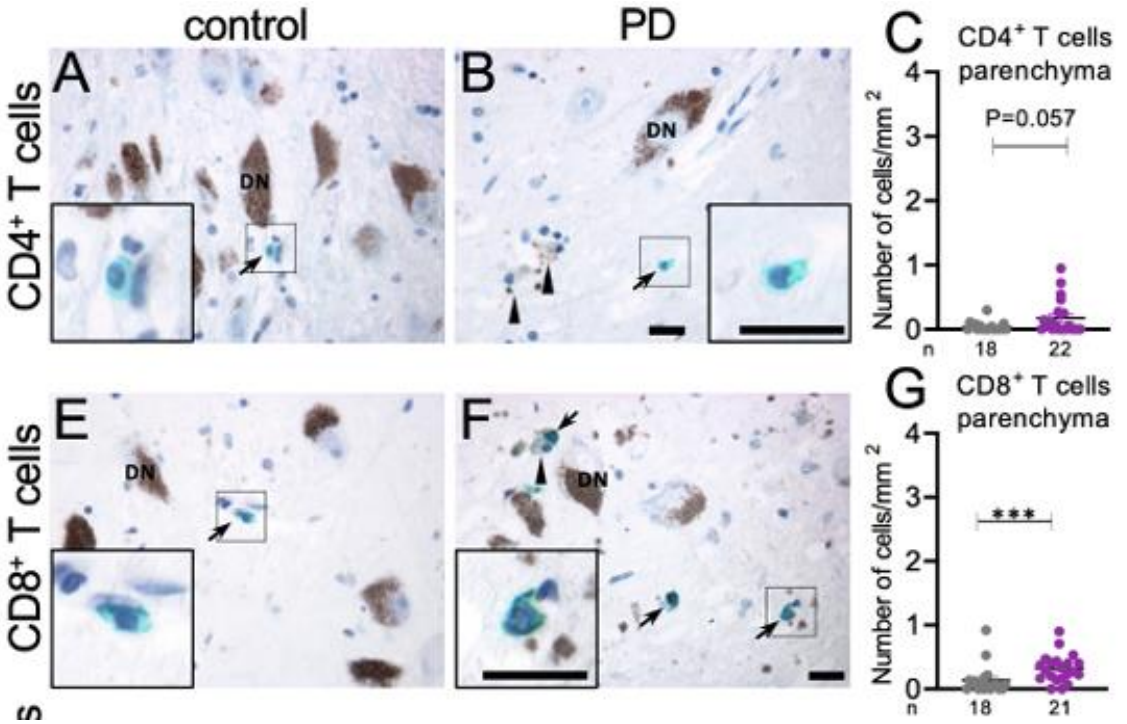
Example: Immune system in Parkinson's disease



Von Herrmann et al., 2018



Karikari et al., 2022

Innate and adaptive immune systems are activated in people with PD

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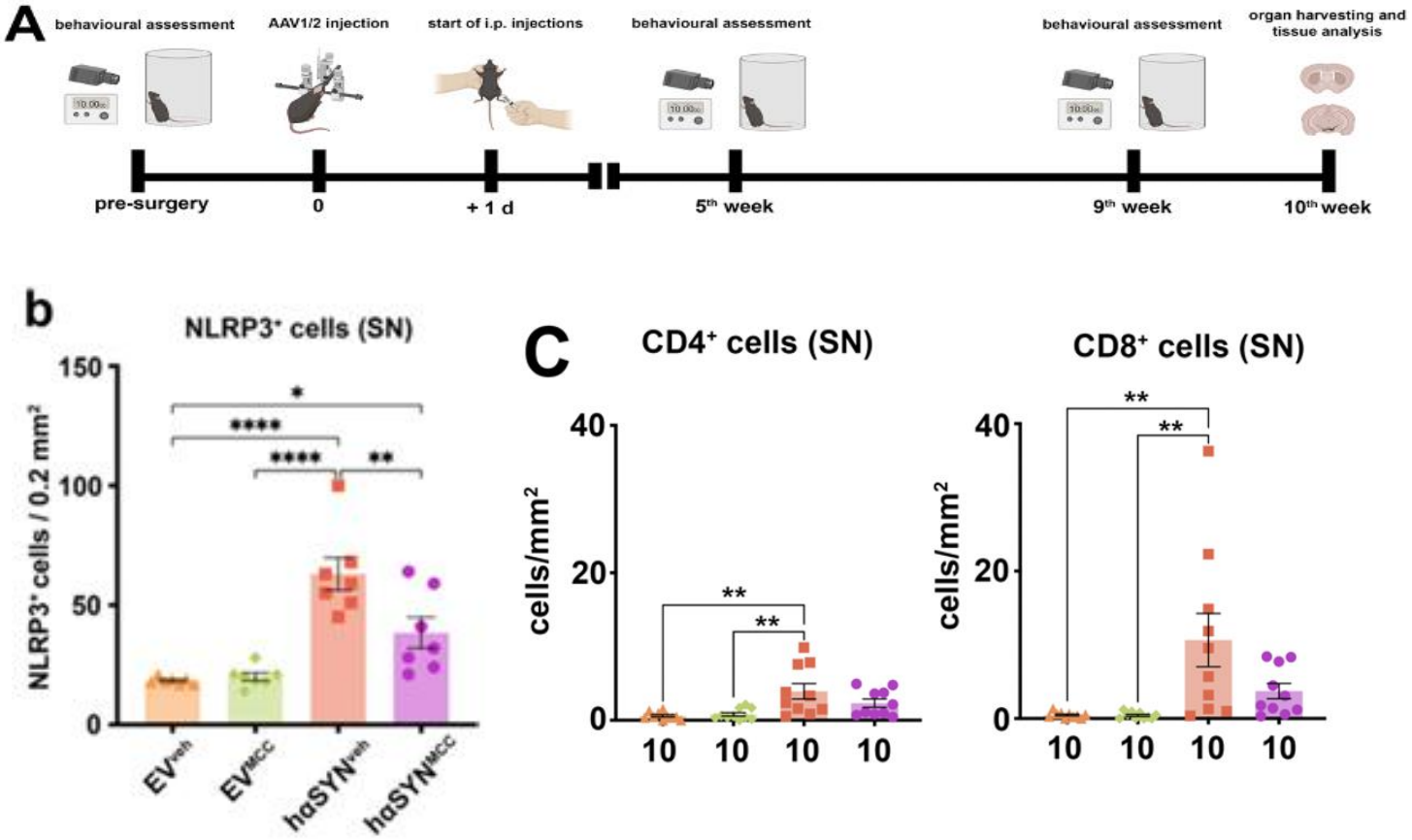
Example: Immune system in preclinical model of Parkinson's disease



Grotemeyer et al., 2023

- Model leads to activation of both innate and adaptive immune system
- Inhibition of NLRP3 (MCC950) reduced NLRP3+ve, CD4+ve and CD8+ve cells in the SN
- MCC950 also protected dopaminergic neurons

Key findings:

- The model broadly replicates changes to the innate and adaptive immune system seen in people with PD
- Blocking these changes via NLRP3 inhibition is neuroprotective
- Is inhibiting NLRP3 neuroprotective in people with PD?

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Example 2: NLRP3 inhibitors clinical data

Inhibitor	Phase	Published / Completion	Findings
VENT-02 (Ventus Therapeutics)	IIa	Ongoing	Not reported
VTX3232 (Ventyx Biosciences)	IIa	Top-line data reported June 2025	VTX3232 reduced inflammatory markers suggesting NLRP3 inhibition was achieved. Clinically significant reductions in MDS-UPDRS Parts II and III were reported
NT-0796 (NodThera)	IIa	Published in 2025	NT-0796 drove reductions in systemic inflammatory markers suggesting NLRP3 inhibition was achieved

- No full Phase II or III trials have been initiated in Parkinson's disease
- Phase IIa data looks promising so far

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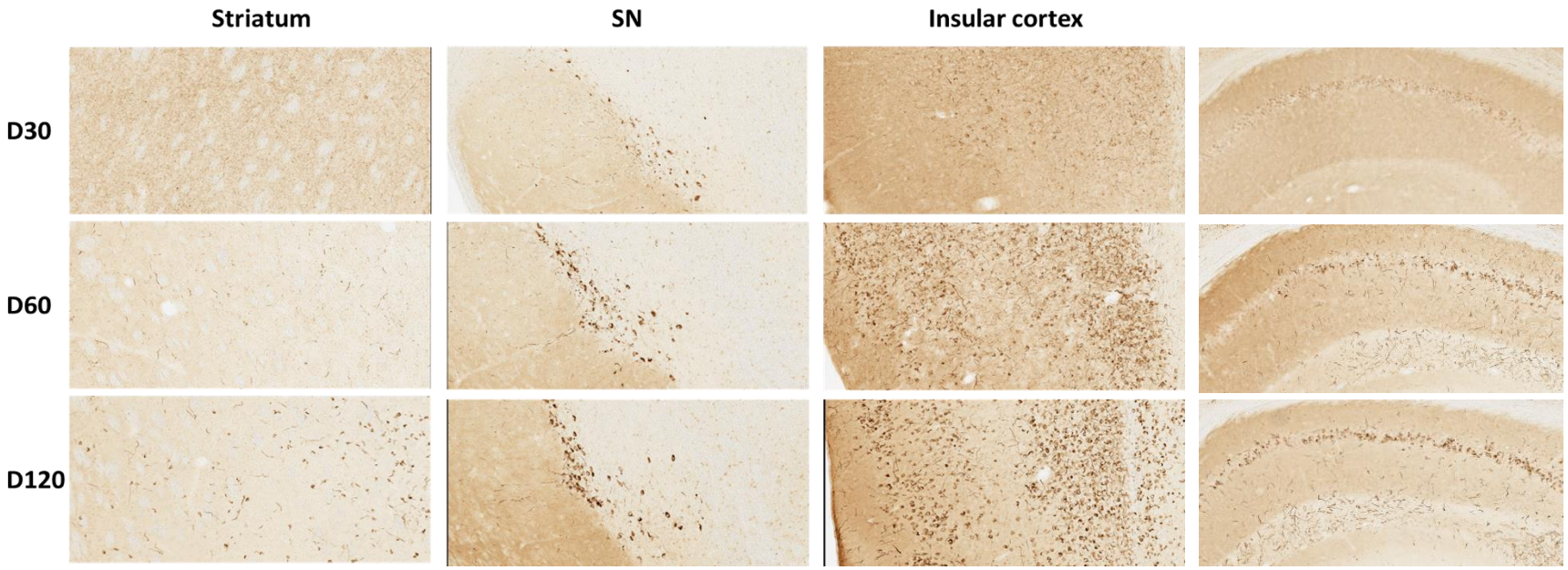
Improvement 3: Robustness and repeatability — aSyn PFF model



aSyn PFF model

- aSyn PFFs are injected into the striatum
- Over time aSyn pathology is seen in areas connected to the striatum
- Seeding model whereby misfolded aggregates of aSyn trigger the conversion of endogenous aSyn into pathological pSer129
- The appearance of pSer129 aSyn in the brain areas that project to the striatum is a finding that is reported in every aSyn PFF paper.
- A good model to examine the effect of therapeutics on aSyn PFF uptake and the conversion of endogenous aSyn to paSyn

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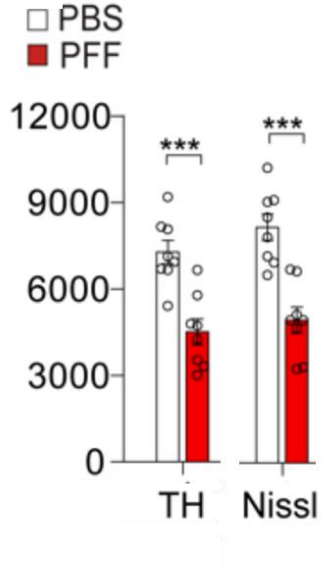
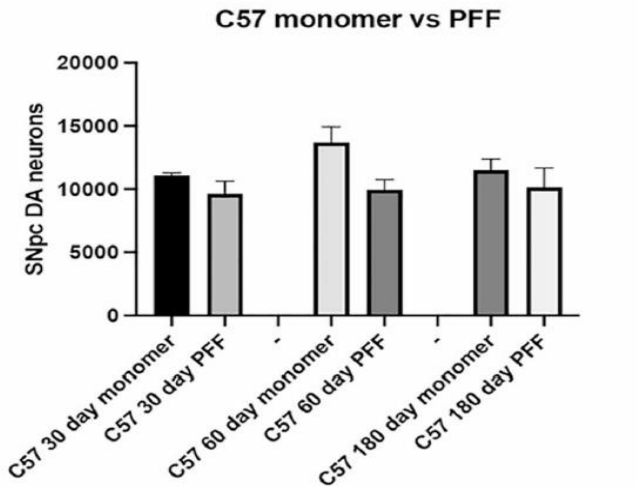
Improvement 3: Robustness and repeatability — aSyn PFF model

- A common, aSyn-driven, model of PD used to evaluate potential therapeutics
- Several iterations, but generally involves direct injection of aSyn PFFs into the striatum





Seo et al., 2024

Toral of 0.1 µg PFF injected into striatum

D180 a **~35% loss of TH+ve cells**

Byrne et al., 2025

Total of 4 µg injected into the striatum

D180 no **significant loss of TH+ve cells (<10%)**

Except for amount of aSyn PFFs injected, similar study designs:

- C57BL/6J mice
- Similar age (Seo: 3-months old; Byrne, 6-12 week old)
- Similar PFF injection co-ordinates
- Killed at 180 days post injection

Both increased pSer129+ve cells in substantia nigra

Significant dopaminergic loss occurred in the Seo study but not in the Byrne study

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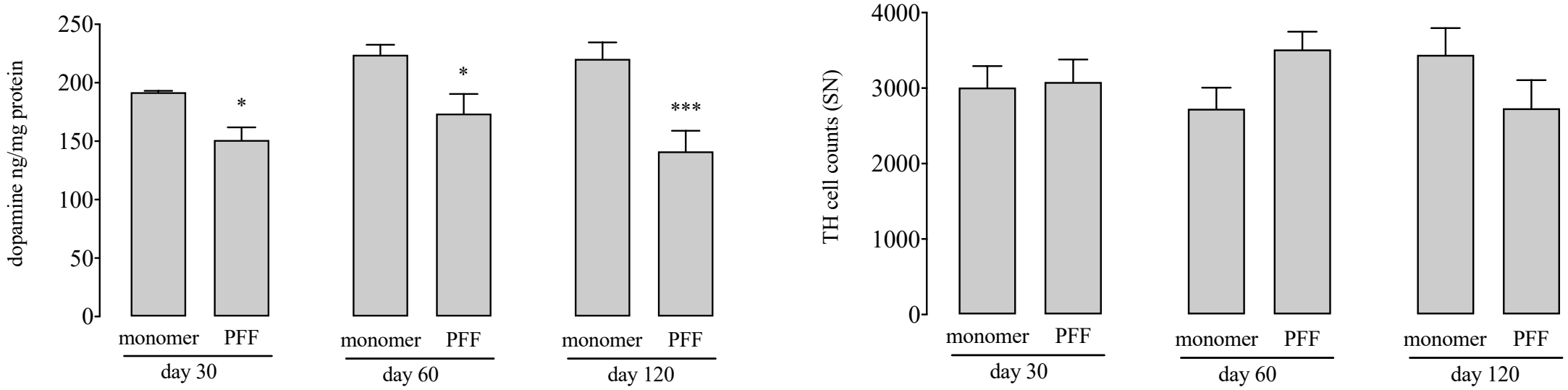
Improvement 3: Robustness and repeatability — aSyn PFF model



Atuka data

- Extensive pSer129 staining in multiple brain regions
- Total of 20 µg PFF injected into each striatum (bilateral model)
- At D120 we observe a loss of striatal dopamine (~30%). No significant loss of TH-positive cells
- Generally, use B6C3 mice but see similar results in C57BL/6J mice

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Improvement 3: Robustness and repeatability – aSyn PFF model

The Challenge

- aSyn PFF models are not as well characterised as neurotoxin (or AAV) models – more potential for variability
- Results may not be easily replicated across labs
- Small differences in methodology (e.g., source of PFF) may be important

The Response

- Need to improve sharing of protocols, SOPs, reagents, etc. across labs
- Working group on how to implement the aSyn PFF model may be warranted
- Quality Management Systems designed to improved reliability and robustness of preclinical data should be implemented

EQIPD Certification

The QMS that Atuka uses is EQIPD certified. EQIPD (Enhancing Quality in Preclinical Data) was specifically designed to boost innovation by ensuring the generation of robust and reliable preclinical data (Bespalov et al., 2021)

EQIPD certified

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Summary

To develop symptomatic therapies, use models that reflect the disease state

To develop disease modifying therapies, use models that reflect the disease process

Symptomatic therapies

- Animal models have had excellent predictive validity for symptomatic treatment of motor symptoms and motor complications.
  - These animal models have focussed on creating a state that accurately replicates the clinical condition

Disease modifying therapies

- No disease modifying therapies have been developed for Parkinson's disease. To improve success we can
  - Reduce heterogeneity in clinical cohorts
    - Implementation of biological classifications of PD may help reduce heterogeneity and define cohorts most likely to respond to given classes of therapeutics.
  - Choose an animal model that recapitulates the process being targeted in the clinical cohort
    - An understanding of what processes a given model does/ does not replicate is critical
    - Incorporate translatable endpoints to demonstrate the desired process is being modulated
    - Good models do not currently exist for all processes though to be important in Parkinson's disease

Robustness and repeatability

- Variability of models across groups has been large, making replication of key results difficult
  - Sharing of information between groups is needed.
  - QMS are needed to reduce bias and improve reliability

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Thank you.