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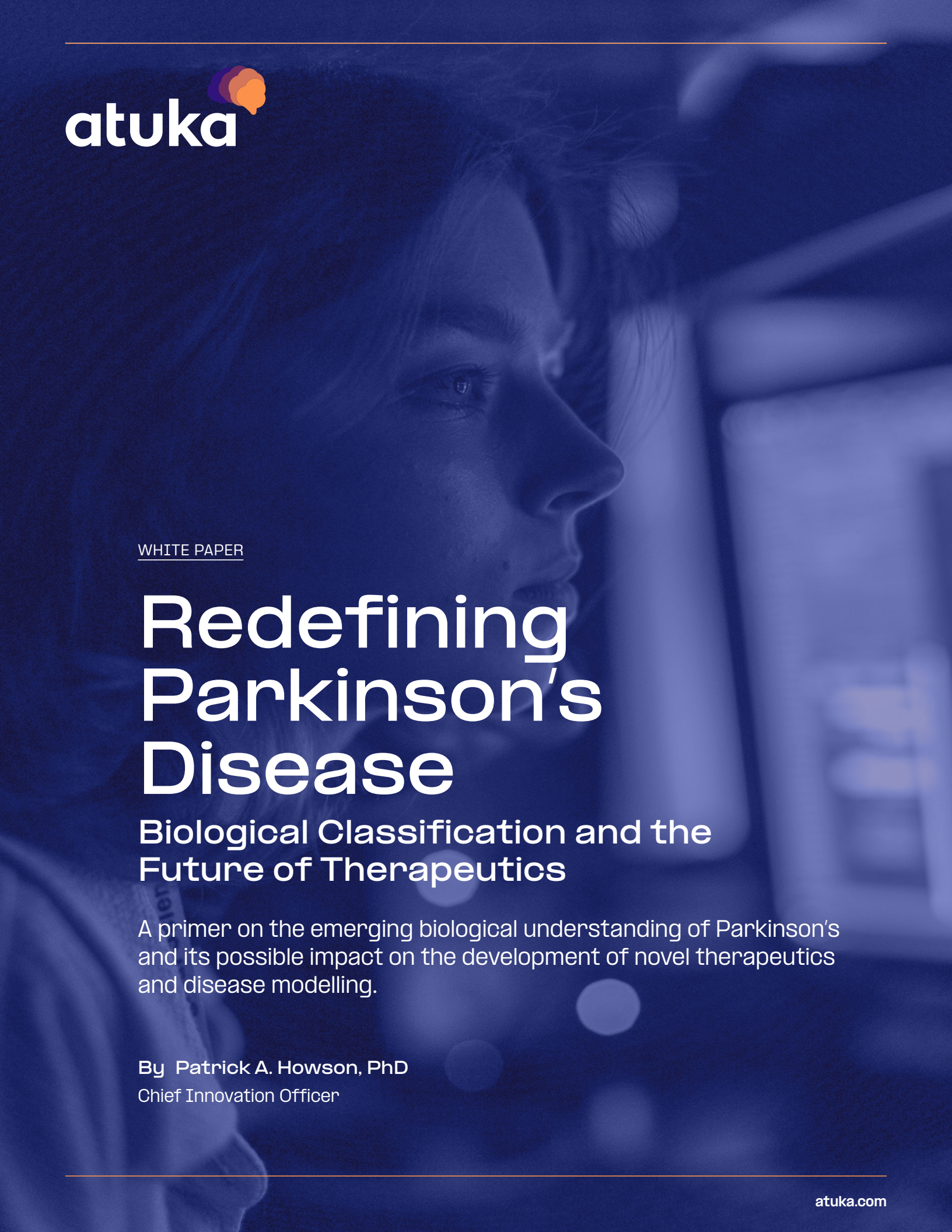

WHITE PAPER

Redefining Parkinson's Disease

Biological Classification and the Future of Therapeutics

A primer on the emerging biological understanding of Parkinson's and its possible impact on the development of novel therapeutics and disease modelling.

By Patrick A. Howson, PhD
Chief Innovation Officer

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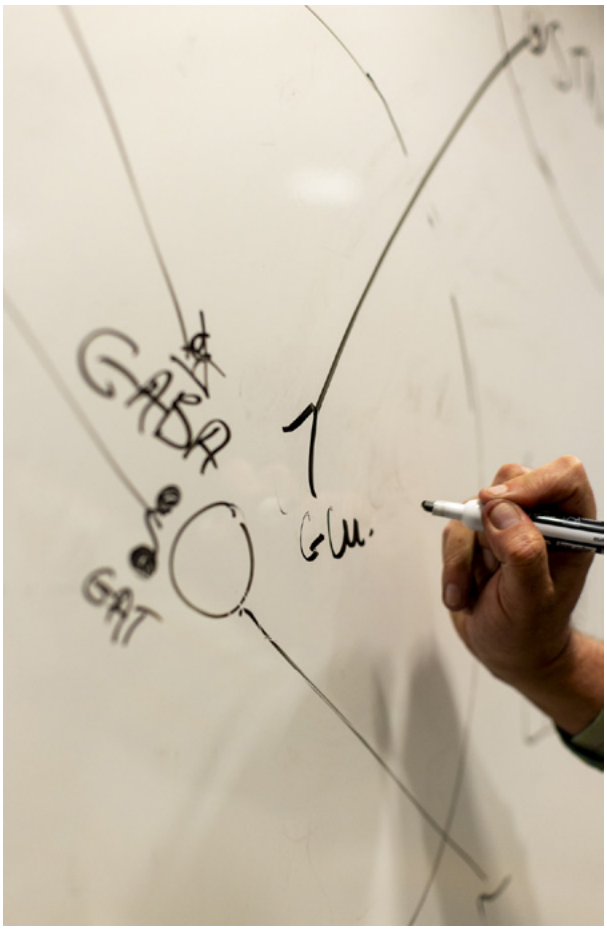
Parkinson's disease (PD) research is undergoing a significant shift from traditional clinical definitions to a biologically based classification system. This change is driven by novel biomarkers developed around a deeper understanding of the disease's complex pathology, particularly in the prodromal (pre–symptomatic) phase.

This phase begins decades before clinical symptoms appear and the disease is diagnosed. Ultimately, this shift is allowing researchers to create a PD framework that will facilitate more comprehensive disease modelling, drive biomarker development, encourage more focused therapeutic targeting, optimize clinical trial design, better align preclinical and clinical research and, ultimately, enable precision medicine—as in medical care designed to optimize therapeutic benefits for an individual.

In this first Atuka white paper on the subject, we will be providing an overview of the current state of biological classifications in PD. We will also consider potential future areas of research that will be used to elaborate, refine, and augment the current biological classifications. We will also briefly discuss the impact that a biological classification of PD may have on preclinical drug development. In future white papers we will explore these topics in more depth.

The need for a biological classification of Parkinson's Disease

Historically, PD and related Lewy body disorders, such as dementia with Lewy bodies (DLB), have been defined primarily by their clinical features. Diagnostic criteria for PD have relied on observable motor symptoms, such as bradykinesia, rigidity, and resting tremor, which typically manifest only after a substantial loss (60–80%) of nigral dopaminergic neurons has already occurred. This reliance on clinical symptoms presents several challenges:



- **Slow diagnosis:** The disease pathology (e.g., α-synuclein aggregation and neurodegeneration) often begins long before symptoms appear, meaning clinical criteria cannot capture these pre-symptomatic or prodromal stages. Thus, performing disease modifying trials in people most likely to benefit, such as those in the earliest stages of the disease is difficult.
- **Heterogeneity:** Clinical syndromes and their progression are highly heterogeneous, leading to overlap among different neurodegenerative disorders. This can lead to the inclusion of people in clinical trials without PD, which increases noise within the data and reduces the power of the trial.
- **Lack of objectivity:** Clinical diagnoses can be subjective and prone to misclassification, as clinical phenotypes may result from different underlying pathologies. As above, this can lead to the inclusion of people in clinical trials without PD, increasing noise and reducing the power of clinical trials.

The exclusive reliance on clinical diagnosis, without adequate biological stratification, is probably a contributing factor to why a disease modifying therapy (DMT) has not been developed for PD. As DMTs aim to interfere with the molecular and cellular mechanisms that lead to neuronal dysfunction and degeneration, a biological understanding of PD is necessary for their development, along with a better understanding of the heterogeneity of biology across people with PD, and how the biology changes as the disease progresses.

A biological classification of PD offers several advantages, including objectivity, homogeneity of patient populations for research, earlier diagnosis (potentially before symptom onset), and patient stratification based on biology, which is essential for

developing targeted precision therapies. Such a system can serve multiple clinical purposes: defining the disease entity, providing diagnosis, stratifying patients into subgroups, and potentially classifying patients according to disease events. Moreover, a deeper understanding of the pathology of PD, how it evolves over time, and the identification of important stages of disease progression also informs several streams of preclinical research such as drug development, target identification, and the development of new animal models of PD.

Proposed biological classification systems for Parkinson's Disease

Two prominent biology-based criteria for PD have recently been proposed: the Synucleinopathy-Neurodegeneration-Genetics (SynNeurGe) framework (Höglinger et al. 2024a) and the Neuronal α-Synuclein Disease (NSD) concept, including the Neuronal α-Synuclein Disease Integrated Staging System (NSD-ISS) (Simuni et al., 2024). Both represent a significant departure from traditional clinical definitions, emphasizing the importance of objective biological markers.

The SynNeurGe research diagnostic criteria

The SynNeurGe framework, proposed by Höglinger et al., aims to provide a broad and flexible biological classification of PD and related Lewy body disorders, acknowledging the disease's inherent complexity and heterogeneity.

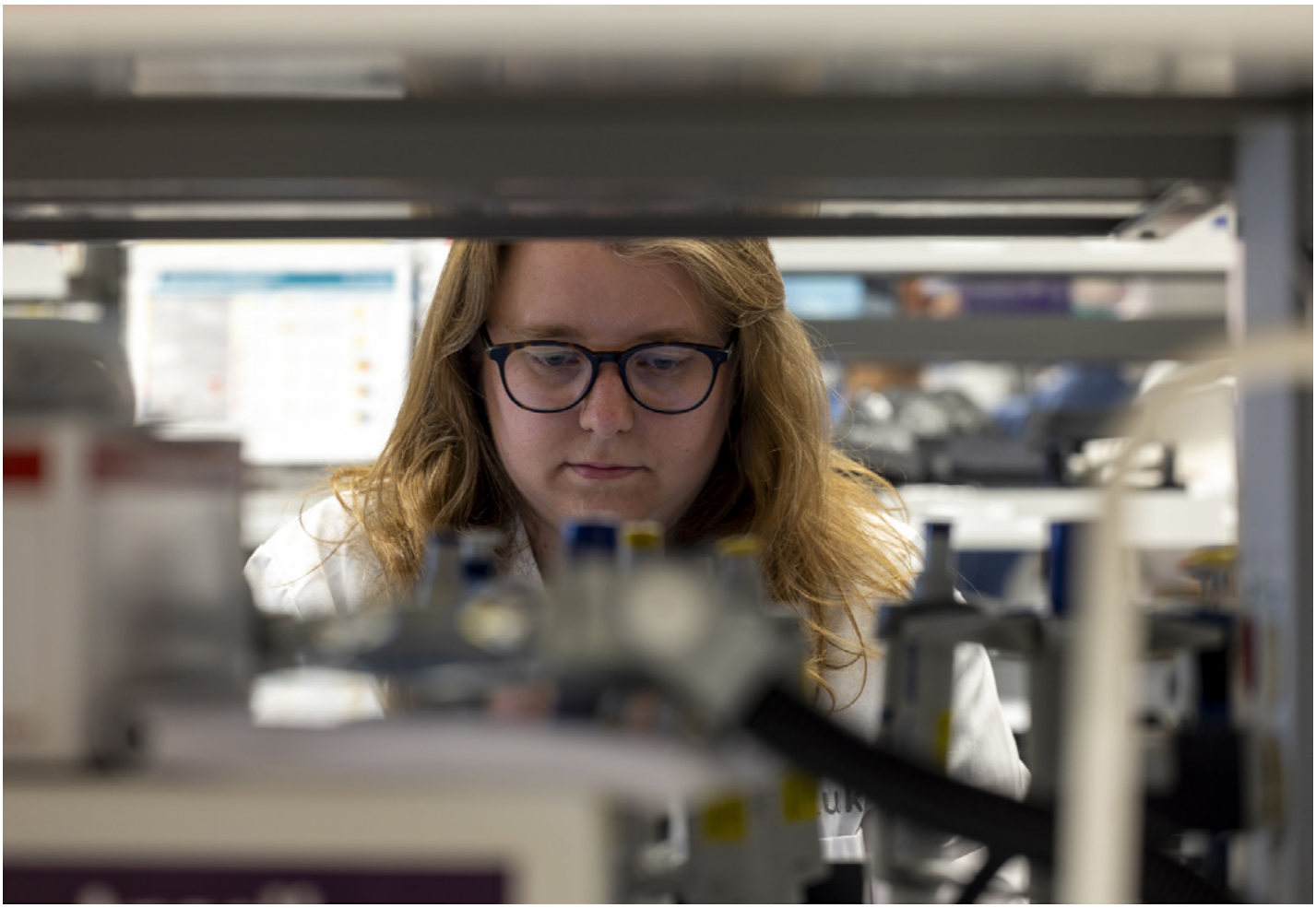
The SynNeurGe system is built upon a three-component biological system, linked to a clinical component:

- S (Synucleinopathy status),
- N (Neurodegeneration status), and
- G (Genetic status)

S (Synucleinopathy status)

Indicates the presence (S+) or absence (S–) of pathological α-synuclein in tissues or cerebrospinal fluid (CSF).

Pathological α-synuclein (misfolded and aggregated) is considered a key molecular anchor of Parkinson's type synucleinopathy,



which forms the basis for the sporadic PD classification. It is the core constituent of Lewy bodies and Lewy neurites, the neuropathological hallmarks of PD and DLB. The presence of aggregated α-synuclein is detected by:

- **Seed Amplification Assays (SAA):** These assays have revolutionized the detection of pathological α-synuclein in vivo, particularly in CSF and skin, with high sensitivities (>80%) and specificities (>90%). The CSF α-synuclein SAA has >90% sensitivity and almost 100% specificity for detecting PD and DLB. It is present in prodromal cases with abnormal dopaminergic imaging and predicts conversion to a clinically diagnosed neurodegenerative parkinsonian syndrome in at-risk individuals.
- **Skin Immunohistochemistry (IHC) or Immunohistofluorescence (IHF):** These methods can detect pathological α-synuclein in skin biopsies with moderate sensitivity (>70% and ≤80%) and high specificity

(>90%). The pattern and distribution of α-synuclein in skin biopsies can also help differentiate PD from multiple system atrophy (MSA).

- **Investigational Biomarkers:** Future promising measures include SAA in other matrices like blood and peripheral tissue, disease-specific post-translational modifications of α-synuclein, and exosome-derived α-synuclein biomarkers. Development of α-synuclein imaging tracers for topographical in vivo detection is also a major research goal.

N (Neurodegeneration status)

Provides evidence of underlying neurodegeneration as defined by neuroimaging procedures.

Characterized by neuronal loss and gliosis, often associated with microglial activation. Neuroimaging techniques are currently the primary approach for direct evidence of neurodegenerative changes, particularly in the nigro-striatal dopamine system. Neurodegeneration is detected by:

- **Dopaminergic Imaging (PET/SPECT):** Includes tracers for dopamine transporter (DAT), L-aromatic acid decarboxylase (F-DOPA), or vesicular monoamine transporter type 2 (VMAT2). These are highly sensitive in detecting reduced nigro-striatal dopamine nerve terminals. While effective at differentiating degenerative parkinsonism from healthy controls or essential tremor, their specificity for distinguishing PD from atypical parkinsonism (e.g., MSA or PSP) is limited.
- **Metabolic FDG-PET:** Reveals functional changes in glucose metabolism (PD-related pattern) that are sensitive to early loss of striatal dopamine innervation. This method has high specificity in differentiating PD from other parkinsonian disorders like MSA or PSP, which exhibit distinct metabolic patterns. It can even detect changes in prodromal disease (e.g., REM sleep behavior disorder).
- **Cardiac Meta-iodobenzylguanidine (MIBG) SPECT:** Detects loss of post-ganglionic sympathetic innervation, indicative of peripheral cardiac sympathetic denervation. This can be seen in prodromal disease. While its specificity for PD is high, it is not perfect, as abnormalities can occur in PSP and MSA.

Possible future markers of neurodegeneration include iron-sensitive MRI, free water imaging, and neuromelanin-sensitive MRI. Multimodal MRI techniques combining various MR contrasts and analyses also show promise for enhanced sensitivity and specificity.

G (Genetic status)

Documents the presence (G+) or absence (G–) of pathogenic gene variants that cause or strongly predispose to PD. This component is characterised by gene penetrance:

- **GF+ (Fully Penetrant):** Includes SNCA triplications and missense variants, and biallelic PRKN, PINK1, and PARK7 variants. Carriers of these variants, by definition, qualify for a diagnosis of genetic PD, regardless of S or N status.
- **GP+ (Strong or Intermediate Predisposition):** Includes SNCA duplications and pathogenic variants in LRRK2, VPS35, and CHCHD2 (strong predisposition), and severely pathogenic GBA1 variants (intermediate predisposition). These variants require additional evidence of neurodegeneration (N+) to be classified as genetic PD.
- **G– (Genetically Indeterminate):** Refers to genetic variants with low predisposition, polygenic risk scores, or absent/unknown genetic contributions. In G– individuals, an S+ and N+ status is required for a biological PD diagnosis.

Some genetic forms of PD (e.g., PRKN, LRRK2 variants) may lack α-synuclein aggregation (S– status), which is explicitly acknowledged in SynNeurGe. This contrasts with NSD's more restricted view (see section on NSD).

C (Clinical status)

In addition to the three components described above, clinical status is also defined by either a single high-specificity clinical feature or multiple lower-specificity clinical features. Thus, once a biological definition (S+, N+, or G+) is established, clinical signs and symptoms (C+) are documented to determine if they are attributable to the underlying biological process of PD.

- **C– (Asymptomatic):** No clinical signs or symptoms potentially associated with PD.
- **Cposs+ (Possibly Related to PD):** Requires at least one clinical feature, which can be from one of several categories; motor, sensory, autonomic, sleep, or cognition. If an individual is just G+ (S– and N–), at least one feature from two categories is needed.
- **Cprob+ (Probably Related to PD):** Requires at least one feature from at least two of the Cposs+ categories (if S+ or N+), or at least one feature from three Cposs+ categories (if only G+). Alternatively, a single, high-specificity features such as clinical parkinsonism (bradykinesia plus rigidity or rest tremor), dementia, polysomnography-confirmed REM sleep behavior disorder, or laboratory-confirmed neurogenic orthostatic hypotension is sufficient.

Temporal relationship

Pathological α-synuclein aggregation is generally believed to precede dopaminergic neuron loss. The proposed staging system is agnostic to the specific localization and spread of synuclein (e.g., "Body-First" vs. "Brain-First" progression), and in vivo imaging agents are critically needed to investigate α-synuclein spread in vivo.

The Neuronal α–Synuclein Disease Integrated Staging System (NSD–ISS)

The NSD-ISS, proposed by Simuni et al., defines neuronal α-synuclein disease as the presence of pathological α-synuclein (S) and stage-dependent evidence of dopaminergic neuronal dysfunction (D). It defines a staging system rooted in these biological anchors and the degree of functional impairment. Like SynNeurGe, NSD-ISS is currently intended exclusively for research use. In essence, the NSD-ISS describes a subset of people that is described by the SynNeurGe classification.

The NSD-ISS uses S and D as primary biological anchors, with genetic status (G) playing a supporting role, and functional impairment defining later stages.

S (Neuronal α–Synuclein Anchor)

This indicates the presence (S+) or absence (S–) of disease-defining pathological (misfolded and aggregated) neuronal α-synuclein. S+ is the fundamental defining feature of neuronal α-synuclein disease.

- **Biomarkers:** Currently, the CSF α-synuclein seed amplification assay (SAA) is the only biomarker considered to have undergone robust validation with high accuracy in multiple independent cohorts.
- **Future Directions:** Development of quantitative SAA measures and imaging tracers for topographical in vivo detection of α-synuclein are major goals. Other promising methods include SAA in blood and peripheral tissue, and immunohistochemical detection of phosphorylated α-synuclein in skin biopsies.

D (Dopaminergic Neuron Dysfunction Anchor)

This indicates the presence (D+) or absence (D–) of dopaminergic dysfunction/degeneration. The degeneration of substantia nigra dopaminergic neurons is a core pathological feature and the second key anchor.

- **Dopamine Transporter (DAT) Imaging:** SPECT imaging using ioflupane (I^{123}) is the most widely used tracer, showing

striatal changes. DAT loss often precedes functional impairment, and reduced DAT binding predicts motor and cognitive progression. A goal is to harmonize DAT imaging outcomes to a single quantitative scale that enables quantitative measures of dopaminergic dysfunction.

- **Other Imaging Modalities:** PET tracers targeting dopamine transporters or vesicular monoamine transporters, and neuromelanin-sensitive MRI are emerging tools.
- **Future possibilities:** The long-term vision is to incorporate biomarkers reflecting neurodegeneration beyond the dopaminergic system, as neuronal α-synuclein disease is multisystemic.

G (Genetic status)

Identifies individuals with genetic variants that cause or increase risk for neuronal α-synuclein disease.

- **Fully penetrant pathogenic variants in SNCA** are currently the only genetic cause sufficient for a diagnosis of neuronal α-synuclein disease.
- **Risk Variants:** Other genetic variants (e.g., GBA1, LRRK2) identify individuals at increased age-dependent risk, but these individuals do not have neuronal α-synuclein disease unless they show evidence of S+.
- **S– D+ G+ Cases:** The NSD-ISS notes that some individuals with pathogenic LRRK2 or PRKN variants may have dopaminergic dysfunction (D+) and parkinsonism but lack α-synuclein pathology (S–). These individuals do not have detectable neuronal α-synuclein disease by NSD-ISS criteria and must be defined and staged separately.

Functional impairment

Functional impairment defines disease progression in later stages of the disease, as it progresses beyond subtle signs/symptoms.

- **Qualitative Progression:** Functional impairment is conceptualized qualitatively as progressing along a

continuum from slight to severe, increasingly impacting activities of daily living.

- **Data-Driven Anchors:** Definitions and specific functional anchors for stages 3–6 (see staging system below) still need to be developed using data from prospective cohort studies and clinical trials (e.g., MDS-UPDRS Parts I and II).

Stages of the NSD–ISS

Unlike the SynNeurGe classification, the NSD-ISS defines discrete stages along the disease continuum, with the biological definition being key for early stages:

- **Genetic Risk Categories:** Individuals with pathogenic variants who do not yet have evidence of pathological α-synuclein are categorized as low (RL) or high (RH) genetic risk. They do not have neuronal α-synuclein disease.
- **Stage 0:** Defined by the presence of a fully penetrant SNCA variant (G+) without clinical signs, symptoms, or functional impairment, and no biomarker evidence of S or D.
- **Stage 1A:** Defined by the presence of pathological α-synuclein (S+) without dopaminergic dysfunction (D–) and no relevant signs or symptoms or functional impairment.
- **Stage 1B:** Defined by the presence of pathological α-synuclein (S+) and dopaminergic dysfunction (D+) but no relevant signs or symptoms or functional impairment.
- **Stage 2 (2A and 2B):** Marked by the presence of subtle clinical signs or symptoms without functional impairment. These can be motor or non-motor.
- **Stages 3–6:** Defined by the severity of functional impairment, progressing from slight (Stage 3), mild (Stage

4), moderate (Stage 5), to severe (Stage 6). These stages are driven by the cumulative effect of motor, cognitive, or other non-motor clinical signs or symptoms.

In the NSD-ISS, neuronal α-synuclein disease is seen as a continuum, where an individual in a stage is presumed to have passed through all preceding stages, starting with Stage 1A. However, progression might not be linear and some individuals may never progress to later stages.

Similarities and differences between SynNeurGe and NSD–ISS

SynNeurGe and NSD-ISS represent a fundamental shift towards the biological classification of PD. In essence, the NSD concept is a more restricted definition of PD that largely fits as a sub-entity within the broader SynNeurGe classification. SynNeurGe aims for a more inclusive and flexible framework that can evolve with future scientific advances, acknowledging the full spectrum of PD's biological heterogeneity, including non-α-synuclein-driven forms, and a broader range of biomarkers for neurodegeneration. The similarities and differences between these classifications have recently been discussed (Höglinger et al. 2024b).

Commonalities

- **SNCA Variants:** Both consider fully penetrant pathogenic variants in SNCA sufficient for a diagnosis of PD/neuronal α-synuclein disease.
- **α-Synuclein SAA in CSF:** Both endorse CSF α-synuclein SAA as a sensitive and specific method to detect pathological α-synuclein aggregates in the CNS.
- **DAT Imaging:** Both accept DAT imaging as a modality to demonstrate nigrostriatal dopaminergic neurodegeneration associated with PD.
- **Research Use Only:** Both explicitly state their criteria are intended for research purposes only, not for routine clinical practice.
- **Focus on Early Intervention:** Both aim to improve early diagnosis and facilitate the development of DMTs that can intervene before significant symptoms or widespread neurodegeneration occur.

Differences

- **Terminology:** SynNeurGe retains the established terminology of "Parkinson's disease" (or DLB) and introduces "Parkinson's type (or Lewy-type) synucleinopathy" for the prevailing Lewy pathology. NSD-ISS introduces the novel term neuronal α-synuclein disease (NSD) to unify PD and

DLB. It restricts the definition of the disease to α-synuclein pathology, essentially viewing it as one disease caused by one biology.

- **Aims and Applicability:** SynNeurGe aims to enable a broad spectrum of future research (epidemiology, genetics, neuroimaging, biomarkers, clinical trials) by providing a classification system that defines subtypes within the wide spectrum of PD. It explicitly accommodates S– forms of genetic PD. By contrast, NSD-ISS is primarily conceptualized for therapeutic trials in early stages of sporadic PD, potentially not fully facilitating targeted approaches in genetically determined forms of PD (e.g., PRKN, LRRK2, GBA1 variants) that might not involve α-synuclein pathology.
- **Methodology:** SynNeurGe was developed through an evidence- and consensus-based approach by academic experts covering various domains of PD. NSD-ISS was developed from a consensus process involving industry representatives and regulatory bodies, with a strong focus on drug approval processes and clinical trial design.
- **Biomarkers Endorsed for S Status:** SynNeurGe endorses both CSF and skin SAA, as well as skin immunohistochemistry/immunofluorescence for detecting synucleinopathy. NSD-ISS only endorses CSF SAA only as the validated method for S status.
- **Biomarkers Endorsed for N Status:** SynNeurGe endorses all presynaptic dopaminergic imaging modalities (DAT SPECT/PET, F-dopa PET, VMAT2 PET), PD-related metabolic pattern in FDG-PET, and MIBG SPECT for demonstrating peripheral autonomic neurodegeneration. By contrast, NSD-ISS currently proposes to only use presynaptic nigrostriatal dopaminergic imaging (mainly DAT scan).
- **Genetic Considerations:** SynNeurGe includes both genetic and sporadic PD, stratifies pathogenic gene variants by their penetrance (fully penetrant vs. predisposition), and acknowledges S– PD in cases not associated with α-synuclein pathology (e.g., some PRKN variants). NSD-ISS focuses on sporadic PD, accepting only very rare SNCA variants as disease-defining Stage 0. It does not formally include other genetic forms of PD unless S+ and D+ are present.
- **Staging:** SynNeurGe refrains from proposing formal disease stages, arguing that longitudinal studies are required

to prove the temporal order of events. It instead focuses on biological classifications or states. NSD-ISS proposes seven stages (0–6), defined by biological markers (S, D), genetic status (SNCA for Stage 0), and increasing degrees of functional impairment. It posits a sequential progression through these stages.

- **Clinical Criteria:** SynNeurGe has well-defined clinical criteria (Cposs+ and Cprob+), characterizing features possibly and probably related to PD, and emphasizes clinical judgment and consistency with early PD. NSD-ISS emphasizes functional impairment (slight, mild, moderate, severe) as the primary clinical anchor for later stages.
- **Implications for asymptomatic S+ Individuals:** SynNeurGe designates S+ individuals without N+ as Parkinson's type synucleinopathy, acknowledging the uncertainty of conversion to clinical PD. It emphasizes the need for N+ confirmation before a diagnosis of disease is made. NSD-ISS labels S+ individuals as Stage 1A NSD, implicitly considering them as having the disease. This is despite acknowledging that some S+ individuals may never develop symptoms.

Continued development of biological classifications of Parkinson's disease

The proposers of both classifications clearly see the current classifications as frameworks which, over time, will be added to and refined. Some of these refinements, such as the development of α-synuclein imaging ligand, are discussed in the Höglinger and Simuni papers. Other potential future developments may include:

- **Refinement of existing anchors.** The way the current anchors (synuclein, neurodegeneration, and genetic status) are used is being constantly refined. For instance, assays have been developed that allow the quantification of aggregated α-synuclein (digital SAA) and α-synuclein ligands that allow the in vivo imaging of α-synuclein pathology continue to be developed and refined. Moreover, new genetic risk factors are being discovered that will enhance our understanding of genetic status. These types of refinement will be easily incorporated into the existing frameworks.
- **Development of non-invasive biomarkers.** Currently, assays that detect pathologic α-synuclein of dopaminergic loss are invasive or require access to specialised equipment; for instance, the SAA assay requires a CSF sample. Biomarkers that rely on blood, saliva, or urine would have the major advantage of being widely available and be able to be processed centrally, thus making an early diagnosis much more practical.
- **Development of new anchors.** Whilst synuclein aggregation and dopaminergic cell loss are undoubtedly characteristics of PD, there are other aspects of PD for which there are currently no accepted biomarkers. Thus, it is likely that biomarkers based around mitochondrial function, immune function, and neuroinflammation will be developed over time.
- **Collecting more longitudinal data in the preclinical phases of the disease.** One of the most well studied cohorts of people with PD is the Parkinson's Progression Markers Initiative (PPMI) cohort. This initiative enrolls prodromal and recently diagnosed people with PD, however, apart from people with a genetic risk factor, the prodromal group had an inclusion criteria of an already existing DAT deficit, thus the earliest, prodromal cohort (those who are S+, D-,

G- in the NSD-ISS classification) are not well defined in this study. Recruitment of people who are S+, D-, G- is ongoing, although it will be several years before a rich dataset is available.

- **Correlation of biomarkers with disease progression.** To-date, little work has been performed correlating changes in biomarkers with disease progression, although studies have suggested that loss of striatal DAT, and CSF levels of l-aromatic acid decarboxylase (also called DOPA decarboxylase), correlates with disease progression, as measured by the UPDRS. Understanding if, and how, pathologic α-synuclein correlates with disease progression will be key to developing therapies aimed at reducing the burden of pathologic α-synuclein.
- **Development of more sensitive ways of tracking disease progression.** The clinical rating scales that are currently used to track disease progression, such as the UPDRS, are relatively insensitive to tracking disease progression in the earliest stages of the disease. Thus, it may be necessary to develop new tools that can track changes in the earliest symptoms of disease. These tools may include refined rating scales, phone apps, voice recognition software, and devices that measure subtle motor changes, e.g., smart pens.
- **A more nuanced understanding of the prodromal features of Parkinson's disease.** A clinical diagnosis of Parkinson's disease is primarily based on a combination of a patient's medical history, a neurological examination, and a review of their symptoms. This can be supplemented by biomarker tests, outlined in this document, that can provide supporting evidence of Parkinson's disease or rule out other conditions, such as a stroke. The clinical criteria, on which a diagnosis is made, include bradykinesia, resting tremor, and rigidity. Thus, by definition, people with a clinical diagnosis of PD will have some symptoms in common, presumably reflecting nigrostriatal pathology. By contrast, people with prodromal PD may have a much more diverse set of symptoms, possibly reflecting a spectrum of pathologies in different areas of the nervous system. Thus, there are multiple prodromal phenotypes which, at least for major ones, e.g. body-first and brain-first, may need characterizing.

- **A deeper understanding of the pathological processes that occur as the disease progresses.** Currently, little is known about how the pathology changes as the disease progresses. For instance, multiple types of programmed cell death have been shown to occur in PD, but whether different types of cell death predominate in different stages of the disease or differs between different brain areas is unknown. Whilst there are some data available from people after they are diagnosed with PD, there is little known about these mechanisms in the prodromal stages of the disease. For therapeutics targeting specific processes, such as pyroptosis, it may be critical to define therapeutic windows when the therapy can be best targeted. Moreover, a deeper understanding of the pathology throughout the disease will strengthen a future staging system and make the system more objective.

Use of biological classifications in research

Both SynNeurGe and NSD-ISS explicitly state their criteria are intended for research purposes only, not for routine clinical practice, so how are the classifications likely to be used in research? A commonality of both classifications is the early identification of people with PD. Thus, both systems can be used to produce more homogeneous clinical trial populations by the removal of individuals who likely do not have early-stage PD. The NSD-ISS classification goes further in this respect than SynNeurGe; indeed, NSD-ISS purposefully excludes some individuals who do have PD, e.g., individuals with LRRK2 or Parkin mutations that do not have a positive SAA test, to generate a more homogenous clinical trial population. There are advantages and disadvantages to such as approach. An NDS-ISS defined cohort is more homogeneous, which may mean that the disease progresses more uniformly, as well as respond more similarly to treatment, and ultimately allow smaller clinical trials to detect therapeutic effects. However, it is unlikely to be useful for LRRK2 or Parkin targeting therapeutics.

The SynNeurGe classification, encompassing all people with PD, may have utility in identifying additional biological anchors across all people with PD. For instance, in contrast to synucleinopathy, inflammation and mitochondrial impairment are thought to occur in all people with PD. In addition, by encompassing all people with PD, it may be better at classifying the diversity of prodromal phenotypes known to occur in PD.

Of course, both classifications can be used alongside each other. An example could be a clinical study that enrolled all individuals with a SynNeurGe classification of PD. However, there could be a pre-specified endpoint that evaluated therapeutic effect only in people that have neuronal synucleinopathy as defined by the NSD-ISS classification. Such a study could be used to study the impact of a therapeutic on a restricted, homogenous, population (NSD-ISS) whilst still assessing therapeutic effect in all people with PD.

The choice of whether to use one or both classifications in research is likely to be governed by the research question that is being addressed. Moving forward it would seem prudent to collect sufficient data that would allow research participants to be accurately placed in both the SynNeurGe and NSD-ISS classifications, as this will allow a comparison of the two classifications within the same research cohort.

Ethical implications of biological classification for PD

Although potentially transformative, the transition to biological diagnosis, especially in asymptomatic individuals, raises significant ethical concerns:

- **Limited Understanding of Natural History:** The long-term prognosis for many individuals identified as "biologically positive" (e.g., S+ D–) but asymptomatic is not fully understood. Many might never develop clinical symptoms.
- **Lack of Approved Disease Modifying Therapies:** Currently, there are no approved disease-modifying therapies for PD. Diagnosing an asymptomatic individual with an incurable disease can have significant psychosocial and economic consequences without immediate therapeutic benefit.
- **Diagnostic Inaccuracy:** Current biomarkers, while highly accurate, are not 100% specific. A 2–10% "false-positive" rate for predicting manifest disease (as noted for SAA in CSF) is unacceptably high for clinical application in asymptomatic individuals.
- **Commercialization:** With genetic testing and α-synuclein SAA already being marketed to consumers, there is a risk of inappropriate use of these tests outside of research settings, underscoring the urgency for establishing clear research criteria and ethical guidelines.
- **Technological Demands:** The current methods are often technology-heavy (e.g., requiring CSF collection, specialized imaging), which may disfavour low- and middle-income countries.

These concerns are significant, though not insurmountable. Where possible, careful consideration should be given to these issues during the research and development stages of diagnostics and therapeutics to ensure that people receive equitable access to both. These considerations should start as soon as possible—bringing together key stakeholders, including people with PD, caregivers, researchers, clinicians, funders, and regulatory authorities—to address concerns in a timely, empathetic way.

Impact of biological classifications on preclinical research

Whilst it has long been recognized that PD has a long prodromal phase and that the disease affects multiple systems, most preclinical drug-development research in PD has focused on dysfunction in the nigrostriatal dopamine system and the development of motor impairments. In clinical practice, diagnosis of PD is primarily based on the presence of parkinsonism, defined by bradykinesia accompanied by either tremor or rigidity, and the careful exclusion of other conditions that can mimic these symptoms. Moreover, at diagnosis, ~60-80% of dopaminergic neurons in the substantia nigra have already been lost, and it is the loss of these neurons that drives the parkinsonism upon which a diagnosis is made. To model this, for more than 50 years we have employed animal models with significant dopamine deficits, and have not been too concerned with how far the mechanisms employed to generate those models (e.g., 6-OHDA, MPTP, rotenone) aligns with the biology in patients, though the models do have biological plausibility. Thus, it is understandable that much preclinical drug development research, and translational success, has been in strategies focused on replacing the lost dopamine (e.g. dopaminergic agonists) or correcting neural circuit imbalances caused by dopamine loss.

Overall, these models have been incredibly useful for developing of symptomatic therapies aimed at reducing motor symptoms driven by loss of dopaminergic function and treatments aimed at reducing the side effects of dopaminergic therapies (L-DOPA-induced dyskinesia). However, the approaches described above have, so far, proven to be less useful at developing disease modifying therapies. One reason for this discrepancy is likely that the models are very good at modelling the impact of loss of dopaminergic neurons, and thus excellent for developing symptomatic therapies, but are worse at modelling the complex biology that causes neuronal dysfunction and ultimately loss in PD.

Impact on developing disease modifying therapies

In attempts to develop therapies to slow the loss of additional dopaminergic neurons (e.g. disease modifying therapies), the field has tended to focus on dopaminergic neurons (in vitro) or on

animal models where there is a loss of dopaminergic neurons in the substantia nigra (toxin and many α-synuclein-based models). This approach is promising but the aetiology of PD is not homogenous, and the models may not sufficiently reflect this. Thus, there may be multiple biological processes that ultimately lead to dopaminergic neurodegeneration and that models of PD driven by a single factor, such as injection of α-synuclein pre-formed fibrils, may not capture this complex biology.

By moving away from a clinical diagnosis, and towards a biological classification, the richness of the biology underpinning PD will be revealed in more detail. For example, the SAA assay can already distinguish people with PD who have a synucleinopathy and those that do not. As the field develops, additional biological anchors may be added to the classification and may lead to people with PD being characterized not only by their clinical features but also by the biology that has led to their development of PD. This greater understanding of the biology underpinning their PD will hopefully lead to disease modifying therapies based upon an individual's underlying pathology and not solely only on their symptoms e.g., therapies aimed at stopping the spread of aggregated α-synuclein are unlikely to be successful in individuals who are SAA negative.

Currently, the heterogeneity of the biology underpinning PD is understudied in preclinical research. To date, most research has used models where the pathology is produced by a single factor, such as a neurotoxin, virally delivered α-synuclein, or injection of α-synuclein pre-formed fibrils. This is starting to change with multiple studies investigating the intersectionality between factors, such as inflammation and spread, the effect of microbiome on α-synuclein pathology, or how pathology in different brain regions can produce multiple parkinsonian symptoms. However, these studies have generally focussed on integrating factors that are known to increase the risk of PD rather than building models based around the underlying biology.

The move toward a biological classification of PD, and the future tools required to improve the classification, will undoubtably drive a deeper understanding of the different types of disease processes underpinning PD. This will allow us to answer fundamental questions such as how does the biology of PD change as the disease progresses? For instance, we know that α-synuclein can cause neuronal degeneration by multiple pathways and that two of the commonest preclinical models of PD, the AAV- α-synuclein

model and the α-synuclein PFF model, may cause dopaminergic neurodegeneration via different forms of programmed cell death.

Thus, it is possible that, in people with PD, the type of neuronal cell death that occurs may differ based on multiple factors; site of origin of synucleinopathy, length of time since the initiation of the synucleinopathy; composition of underlying co-pathologies, environmental factors, genetic factors, etc. Thus, different therapeutic strategies to protect dopaminergic neurons may be required depending on the underlying biology. Moreover, at the point of diagnosis, people with PD have already lost ~60-80% of their dopaminergic neurons and our knowledge about cell loss in PD is largely based on the mechanism of how the remaining dopaminergic neurons degenerate. Understanding how the initial cell loss occurs may be critical to developing disease modifying therapies, especially if the biological classification of PD can identify people with an ongoing synucleinopathy earlier than currently possible.

Some of the clinical work in this area is already ongoing; PPMI is being expanded to include people with very early synucleinopathy. Over time, this will allow access to more post-mortem tissue from people with a synucleinopathy who are prodromal for PD and a richer understanding of the biology underpinning PD. This will further enable us to define different forms of PD based on their biology (e.g., PD associated with a neuronal synucleinopathy vs. PD not associated with a neuronal synucleinopathy, body first PD vs. brain first PD) and enable us also to answer questions on how the biology underpinning PD changes over time (e.g., is the mechanism of cell death in the earliest, prodromal, stages of PD the same that occurs in late-stage PD; what is the relative importance of inflammation, mitochondrial impairment, synuclein-induced toxicity, and lysosomal impairment at different stages of the disease).

At Atuka, we need to carefully assess the existing, and follow the emerging, clinical data to better understand what aspects of PD our existing animal models best capture, and to identify new models that may be needed to replicate aspects of PD biology that are currently inadequately captured. In doing so we will create a suite of models that can be used to assess the potential of therapeutics and define appropriate clinical studies based on how potential treatments modulate biological aspects underpinning PD. Initially, this will involve:

- Mapping existing animal models to the SynNeurGe and NSD-ISS classifications.

- Identifying gaps for stages where models do not currently exist and generate models to address these gaps.
- Ensuring that the models align with the biology underpinning each classification stage, i.e., that they model the underlying biology and not just the biological anchors.

Currently, there is no consensus on what would constitute a 'fit-for-purpose' prodromal model, nor a strategy of how such models could be validated in the clinic. However, if we are to truly develop therapeutic strategies that will prevent people with an early-stage synucleinopathy from ever developing PD then these issues need to be addressed, and the tools allowing drug-developers to assess therapeutics in appropriate models need to be developed. A deep dive into this topic will be a focus of a subsequent Atuka White Paper that will address questions such as: can we build models based around the current anchors of biological classification (S, N and G); would models based around the biological anchors provide a good approximation of the complex biology of PD; and should models focus on defined aspects of biology known to be affected in PD, such as neurodegeneration or inflammation, rather than generating holistic models of PD.

Impact on developing symptomatic therapies

Animal models of PD, especially the MPTP-lesioned non-human primate, have proven to have excellent predictive validity of the clinical effect, at least with respect to Phase II proof-of-concept studies, of therapeutics developed to address the motor symptoms of PD. These models, where symptoms are essentially driven by loss of dopaminergic signalling in the nigrostriatal pathway, and concomitant circuit abnormalities, are now well-defined. Thus, we might consider the need for additional models of PD to assess symptomatic therapies for motor symptoms as less urgent—at least as long as patients included in Phase II meet the requirement of (D+) as defined in the frameworks discussed above.

Known caveats to this are therapies aimed specifically at motor symptoms that are not well modelled in animals (e.g. tremor), or therapeutic targets that are removed/inhibited in PD but not in animal models. However, we should still consider the impact of a biological classification of PD on developing symptomatic therapies for motor impairment associated with PD. For instance, when developing motor symptomatic therapies, how much does the construct validity of the disease process, over the construct validity

of the circuit abnormality generating systems, impact on the predictive validity of a model? Moreover, can the predictive validity of these models be improved as we refine Phase II populations defined by underlying disease biology. These issues have rarely been discussed and will be the focus for a subsequent white paper in this series.

In developing symptomatic therapies for non-motor symptoms, there may be an even greater need or opportunity to incorporate an understanding of the emerging biology of the disease in the models available to us. Again, these considerations will be discussed in greater detail in a subsequent white paper. But to provide a broad framework in which to consider these questions, let us first recognize the opportunity. In addition to the motor symptoms, it has long been recognised that people with PD also exhibit a wide range of non-motor symptoms (Table 1).

Table 1. Summary of non–motor symptoms experienced by people with PD

NEUROPSYCHIATRIC	COGNITIVE	AUTONOMIC	SLEEP DISORDERS	SENSORY ABNORMALITIES
Depression	Executive dysfunction	Orthostatic hypotension	Insomnia	Hyposmia
Anxiety	Memory loss	Constipation	Somnolence	Pain
Impulsive control disorder	Dementia	Fecal incontinence	Excessive daytime sleepiness	Ageusia (loss of taste)
Psychosis		Nausea	Restless leg syndrome	Numbness
Anhedonia		Vomiting	Sleep attacks	Paresthesia
Hallucinations		Drooling	Periodic limb movements in sleep	
Apathy		Urinary incontinence and urgency	REM sleep behaviour disorder	
Attention deficit disorder		Sexual dysfunction	Vivid dreaming	
Panic attacks		Altered cardiac reflexes		

An individual with PD will not experience all NMS, and there is not a single NMS that is experienced by all people with PD. Moreover, the development of these NMS may be before, alongside, or after the development of motor symptoms. Therefore, the development of NMS in PD is very heterogeneous and it is unknown whether development of a single NMS across people with PD, such as cognitive impairment, is produced by a similar pathology (e.g., neuronal dysfunction within a structure) or disparate pathologies (e.g., across individuals can pathologies in different brain regions result in a common functional impairment to a specific brain

region), nor is it known how they align with staging or classification as defined by biology of disease classification.

Thus, compared to the motor impairment in PD, the biology responsible for the expression of NMS is much less well understood. In some patients, for example, cognitive impairment is likely driven by dopamine loss, (D+) on the frameworks described above, and for those populations we would argue that the MPTP-lesioned NHP is likely a good model to assess therapeutics, as it is for motor symptoms. Furthermore, the MPTP-lesioned macaque has been used to model various NMS, including sleep dysfunction, anxiety, psychosis, apathy, attention deficit disorder, cognitive impairment, and urinary dysfunction; indeed, many of these endpoints have been used to assess potential therapeutics. However, unlike for motor symptoms, the predictive validity of this model for NMS has not been systematically evaluated, and thus the utility of this model in developing symptomatic treatments for NMS of PD is unknown. Given the predictive uncertainty of the MPTP-lesioned macaque with regards to NMS, and the fact that the pathology underpinning the development of NMS in PD may be much more heterogenous that the pathology underpinning motor impairment, the impact of a biological classification of PD on the development of symptomatic therapies for NMS in PD may be larger than that for symptomatic therapies for motor impairments.

Summary

In summary, the proposed biological classifications of PD, SynNeurGe and NSD-ISS, represent a transformative step forward in the field. While differing in their specific scope and philosophical approaches to staging, both acknowledge the critical role of α-synuclein pathology, neurodegeneration, and genetics in defining the disease. By fostering a biologically consistent approach to research, these frameworks aim to accelerate the discovery and testing of disease-modifying therapies, ultimately moving towards a future of precision medicine for individuals affected by Parkinson's disease.

This Atuka White Paper provides an overview of current concepts in the biological classification of PD. In a series of subsequent papers, we will provide more in-depth analysis in this area, which will include covering whether there can be a single biological classification of PD, how a biological classification of PD might alter drug development and animal models of PD, and how biomarker research will help drive the next generation of biological classification frameworks.

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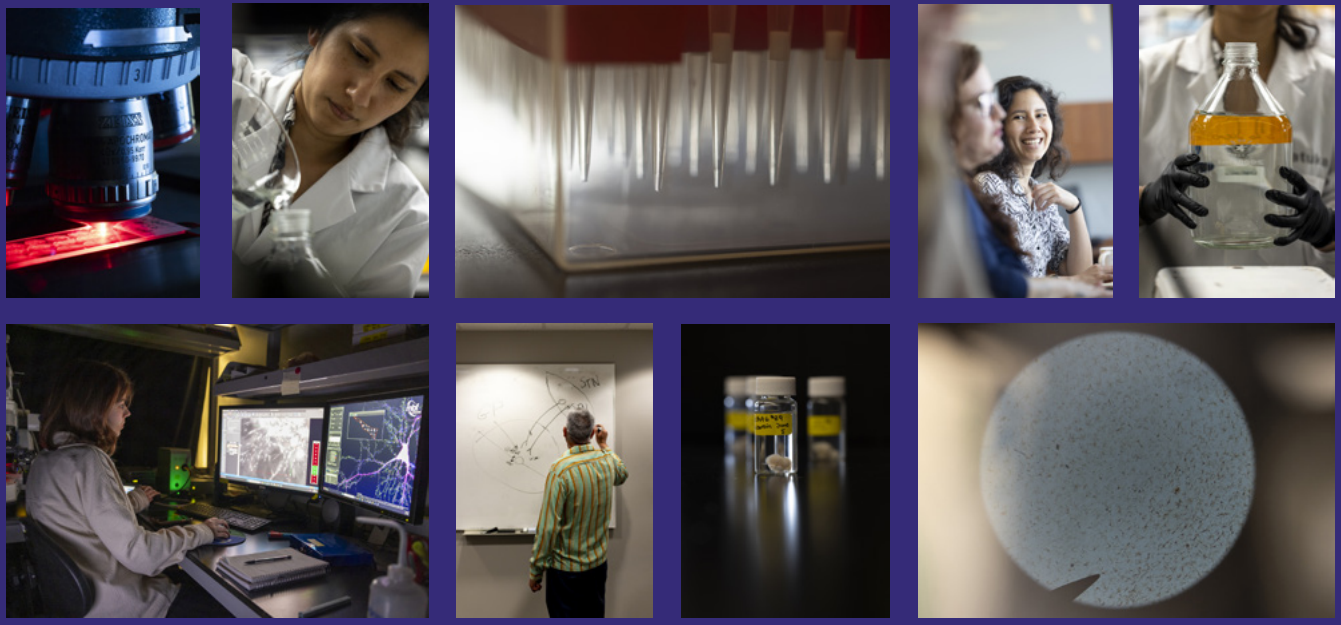
About the Authors

Dr. Patrick Howson's primary research interest is in the development of disease-modifying therapies for neurodegenerative disorders. Patrick has a broad understanding of drug development through several years of working in virtual biotechs, where he has been responsible for research and development projects such as drug-screening programs, manufacture of GMP grade API, IND-enabling studies and Phase I and II clinical trials, including trials in Parkinson's disease. He uses this experience to help our partners develop preclinical programs suitable for their stage of development. Patrick is also experienced in the generation and management of intellectual property and is an inventor of several patents covering therapeutic approaches for Parkinson's disease. He has been with Atuka since 2013 and serves as the company's Chief Innovation Officer.



A cure for Parkinson's, faster, through the world's best preclinical neuroscience.

Atuka's lead scientists have dedicated their careers to furthering our understanding of Parkinson's disease, advancing novel therapeutics, and alleviating the burden of those suffering from neurological disorders.

For more than 20 years, we have collaborated with our partners to provide preclinical services that expand the frontiers of Parkinson's disease research, and help make new, life-changing therapeutics a reality. Our neuroscientists have extensive preclinical experience developing therapies for numerous indications—including Parkinson's, cognitive disorders, Alzheimer's, ALS and other movement disorders such as dystonia and dyskinesia—across multiple modalities, including small molecules and biologics.

Founded by Dr. Jonathan Brotchie in 2003, Atuka has been involved in the preclinical evaluation of more than 300 potential therapeutics, predominantly in Parkinson's disease, of which more than 30 have progressed to clinical trials—a level of experience without equal in our field globally.

Our lead scientists have collectively published more than 300 peer-reviewed, highly-cited papers, and individually possess h-indices ranging from 25 to 70.

Atuka has collaborated with over 90 organizations, including large pharmaceutical and biotech companies, charitable foundations, universities, and government agencies. Over the course of more than 400 preclinical projects, targeting more than 60 mechanisms of action, we have built an extremely rich understanding of Parkinson's disease, its causes, and potential treatments.

With offices and facilities in Toronto and Suzhou, our team is diverse both in background and expertise, bringing to every one of our partner engagements a spirit of close collaboration, along with a commitment to the highest ethical standards in scientific research.

Atuka Inc.
MaRS Discovery District
101 College St
Toronto ON M5G 0A3
Canada

atuka.com

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