

Review

A Proposed Roadmap for Parkinson's Disease Proof of Concept Clinical Trials Investigating Compounds Targeting Alpha-Synuclein

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Abstract. The convergence of human molecular genetics and Lewy pathology of Parkinson's disease (PD) have led to a robust, clinical-stage pipeline of alpha-synuclein (α -syn)-targeted therapies that have the potential to slow or stop the progression of PD and other synucleinopathies. To facilitate the development of these and earlier stage investigational molecules, the Michael J. Fox Foundation for Parkinson's Research convened a group of leaders in the field of PD research from academia and industry, the Alpha-Synuclein Clinical Path Working Group. This group set out to develop recommendations on preclinical and clinical research that can de-risk the development of α -syn targeting therapies. This consensus white paper provides a translational framework, from the selection of animal models and associated end-points to decision-driving biomarkers as

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well as considerations for the design of clinical proof-of-concept studies. It also identifies current gaps in our biomarker toolkit and the status of the discovery and validation of α -syn-associated biomarkers that could help fill these gaps. Further, it highlights the importance of the emerging digital technology to supplement the capture and monitoring of clinical outcomes. Although the development of disease-modifying therapies targeting α -syn face profound challenges, we remain optimistic that meaningful strides will be made soon toward the identification and approval of disease-modifying therapeutics targeting α -syn.

Keywords: Alpha-synuclein, biomarkers, digital endpoints, immunotherapies, animal models, experimental therapies, neuroimaging

INTRODUCTION AND OVERVIEW

Parkinson's disease (PD) is the second most common neurodegenerative disorder, affecting approximately 1.2% of the world population over the age of 70. In the United States, an estimated 630,000 people had PD in 2010. With anticipated demographic changes due to an aging population, and if no disease-modifying treatment is found, the prevalence is expected to reach 930,000 by 2020 and 1.24 million by 2030 [1–3]. Recent years have seen the approval of several new therapies that address motor or non-motor symptoms of PD, however no therapeutic is available yet to slow disease progression.

In 2017, The Michael J. Fox Foundation (MJFF) convened a group of leaders in the field of PD research from academia and industry, the Alpha-Synuclein Clinical Path Working Group. This working group was tasked to develop a strategic consensus and put forth recommendations on preclinical and clinical research directed at the development of alpha-synuclein (α -syn) targeted, potentially disease-modifying therapies for PD. α -syn has become one of the most attractive targets for new therapeutic agents to slow or arrest the progression of PD and other synucleinopathies, including Dementia with Lewy Bodies (DLB), Multiple System Atrophy (MSA), and Pure Autonomic Failure. A detailed discussion on α -syn as a target for PD is beyond the scope of this consensus paper. However, the interest in targeting α -syn stems from the following three converging lines of evidence that implicate a pathogenic role of α -syn in PD: 1) mutations in the gene encoding α -syn, *SNCA*, cause autosomal dominant PD, suggesting that a gain of toxic function may underlie the pathogenesis in familial PD cases (reviewed in [4]); 2) several polymorphic variants at the *SNCA* locus affect the expression of α -syn and are risk factors of sporadic PD, which constitutes over 90% of PD incidence [5–7]; 3) α -syn is the major protein component of Lewy bodies and Lewy neurites, the intracellular inclusions that are the

pathological hallmarks of PD [8, 9]. Based on these observations, proposed therapeutic strategies primarily aim to reduce the pathogenic burden of α -syn by decreasing its production, reducing its aggregation, augmenting its clearance, or neutralizing its toxic effects *via* sequestration (Table 1).

In this regard, α -syn-targeting strategies are heavily informed by the amyloid hypothesis of Alzheimer's disease (AD) that posits, in an analogous manner to the α -syn hypothesis of PD, a toxic gain of function of beta-amyloid ($A\beta$), an extracellular proteinaceous deposit. In recent years, results of multiple clinical studies testing the amyloid hypothesis have emerged, which provide critical insights into designing clinical trials of α -syn targeted therapies. Although some recent early-phase data on $A\beta$ -targeted therapies for AD are encouraging [10], most have not met their primary efficacy end-points or demonstrated clinically meaningful effects in registration trials [11]. Analyses of the $A\beta$ -targeted AD trials that have failed in the past suggest a number of factors contributing to their failures: 1) insufficient evidence of target engagement [12, 13]; 2) inclusion of patients who did not have $A\beta$ pathology; 3) enrollment of subjects at a stage of the disease that may have been too advanced to permit attaining a meaningful clinical benefit; 4) lack of sensitivity of clinical endpoints used; and 5) selective targeting of a single pathogenic mechanism. Finally, an additional option is that $A\beta$ simply is not a valid therapeutic target in sporadic AD [14], raising the mirror-image possibility that α -syn may not be a suitable therapeutic target for non-*SNCA* PD (Espay et al., 2018, submitted).

Could past failures in AD have been prevented or the efforts terminated at an earlier stage to re-deploy resources toward alternative targets through improvement in translational research, biomarker use and clinical trial design? Indeed, emerging data from recent early phase clinical trials of AD indicate that a combination of patient enrichment strategy with biomarkers of $A\beta$ pathology could provide a

Table 1
Proposed therapeutic strategies targeting alpha-synuclein

| Therapeutic Objective and Mode of Action | Therapeutic Examples | Most Advanced Phase of Drug Development Known |
|---|--|---|
| Reduce α -syn levels <i>via</i> inhibition of transcription or translation of the SNCA gene | ASOs, siRNA | Preclinical |
| Reduce/prevent formation of α -syn aggregates* | NPT200-11 ^a | Phase I completed |
| Enhance lysosomal or proteosomal enzyme activity to promote clearance of intracellular α -syn | GZ/SAR402671 ^b | Phase II underway |
| Neutralize/clear extracellular α -syn* and/or inhibit cell-to-cell transmission of α -syn | RO7046015 (PRX002) ^c , BIIB054 ^d | Phase II underway |

ASOs, antisense oligonucleotides; siRNA, small interfering ribonucleic acid. *Presumed toxic species of α -syn. ^aClinicalTrials.gov Identifier: NCT026066. ^bClinicalTrials.gov Identifier: NCT02906020. ^cClinicalTrials.gov Identifier: NCT03100149. ^dClinicalTrials.gov Identifier: NCT03318523.

strong data package upon which a decision to initiate registration trials could be founded with greater confidence [10, 15, 16]. In this paper, we will apply lessons from the AD field as well as prior therapeutic trials in PD and other central nervous system (CNS) disorders, to propose a framework for translational research and early stage clinical development of α -syn-targeted therapies. We posit that in addition to ensuring the validity of the selected therapeutic targets to disease biology and drug-like attributes of an investigational therapy, the following five types of biomarker-based evidence (adapted from <http://www.ncbi.nlm.nih.gov/books/NBK326791/>) generated in both translational animal models and early stage clinical studies could improve success rates (Fig. 1):

1. Target Engagement (TE): a biomarker that indicates that the intervention gets to the site of action and engages its intended target,
2. Pharmacodynamic/Response: a biomarker used to show that a biological response has occurred in an individual who has received an intervention or exposure,
3. Disease Monitoring: a biomarker measured serially and used to detect a change in the degree or extent of disease. For the purpose of this paper, we propose that disease-monitoring biomarkers may be used to generate the so-called Proof of Principle (PoP) or Proof of Concept (PoC) milestones. PoP is achieved when a biomarker is used to indicate that an intervention modifies the known pathology/physiology of PD (e.g., α -synuclein aggregates) whereas PoC is achieved when an intervention produces a clinical response that may be predictive of efficacy in patients. A

go/no-go decision on the basis of a PoP endpoint assumes that the PoP biomarker is predictive of clinical efficacy; a Disease Monitoring biomarker may or may not be the same as one that demonstrates the pharmacodynamic effects of a given drug candidate,

4. Safety: a biomarker used to indicate the presence or extent of toxicity related to an intervention or exposure (the toxicity may be related to either on- or off-target pharmacology of the intervention),
5. Patient Selection/Stratification: one or more biomarkers that can be used in early or late stage clinical trials to select patients who both possess the desired target and are at a high likelihood to respond to the drug candidate within the timeframe of the planned study [17]. These biomarkers could be accepted as Predictive Biomarkers with evidence that the biomarker identifies individuals who are more likely than similar patients without the biomarker to experience a favorable or unfavorable effect from a specific intervention or exposure.

Before trust can be placed in biomarkers for decision-making, however, several factors must be taken into account. These include 1) analytical validation of the biomarker assays, 2) understanding predictive value with respect to the intended utility of the biomarker, and 3) rate of change in the biomarker as associated with pathophysiology or natural history of the disease. Importantly, for pharmacodynamic and disease modification biomarkers, it is imperative to assess and establish that a drug candidate can modify a specific biomarker in careful, dose-ranging animal studies. In the following sections of this white paper, we provide a framework for

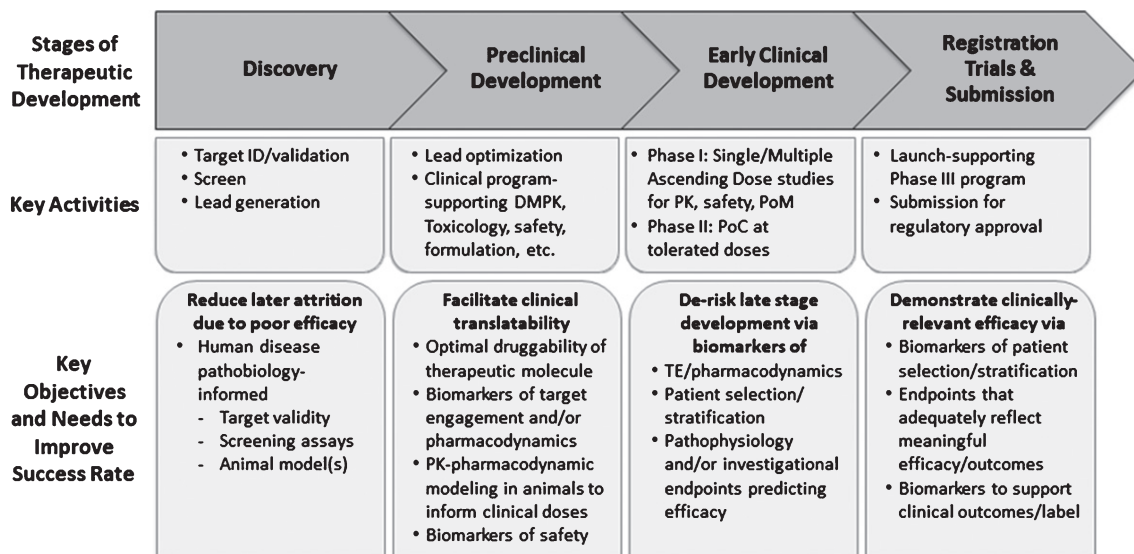


Fig. 1. Key Stages of Therapeutic Discovery and Development. Key stages of therapeutic discovery and development and associated objectives aimed at improving the probability of technical success. Note the specific utility of biomarkers to inform preclinical and clinical decisions during each stage. DMPK, drug metabolism and pharmacokinetics; ID, identification; PK, pharmacokinetics; PoC, proof of concept; PoM, proof of mechanism; TE, target engagement.

selecting an animal model to generate clinically translatable data, specific biomarkers for α -syn-targeted therapies and clinical PoC factors, and planning that integrates not only pathophysiological endpoints but also the emerging technology of wearable devices to monitor clinical outcomes.

TRANSLATIONAL RESEARCH

The current pipeline of α -syn-targeted therapies (Table 1) is comprised of both small molecules and biologics such as nucleic acid analogs, proteins, and gene therapy vectors. Independent of the therapeutic modality, a key translational research element in the early discovery and lead optimization stages (Fig. 1) is the use of animal models that recapitulate relevant aspects of disease pathophysiology. Translational research also aims to deliver clinically translatable biomarkers of TE, pharmacodynamics, and PoP which also inform initial clinical study design, including dose and dosing regimens. Hence, in the animal models and subsequent human studies, it is critical to utilize similar, if not identical, biomarkers and/or endpoint methodologies. The following sections provide an overview of animal models and their relevance to the development of α -syn-targeted therapies.

Animal model studies

Several rodent and non-human primate models of α -syn aggregation exist. It is beyond the scope of this review to describe all existing animal models of α -syn pathology, and readers are referred to recent reviews on the topic [18–24]. In this section, we focus on conceptual challenges as well as the utility of current animal models in α -syn-targeted drug discovery.

Conceptual challenges with current models

A major challenge in the field is the lack of consensus on the specific molecular species of α -syn (e.g., oligomer or fibril and specific strains associated with either, or post-translationally modified α -syn species, etc.) responsible for PD pathogenesis, which engenders uncertainty around the construct validity of the models. In addition, definitive evidence of a particular α -syn species causing a specific synucleinopathy (e.g., PD versus MSA) in humans is lacking, even though animal models are beginning to shed light on strain-specific synucleinopathy [25–28]. In section 3 we detail ongoing studies to develop and test α -syn-targeted biomarkers on human biospecimens to garner insights into α -syn species pathogenicity. These insights will undoubtedly refine the generation and characterization of future animal models.

Currently, no animal model faithfully reproduces all the key clinical features of PD. The predictive validity of an animal model can only be established once an α -syn-targeted therapy is found to be effective in humans. Some have argued that because of these factors, testing in animal models is of limited utility in PD drug development. However, as discussed below, animal models of synucleinopathy are critical to establish *in vivo* proof of mechanism and discovery of translational biomarkers of TE, pharmacodynamics, and PoP for novel α -syn therapies under development.

Commonly used rodent models

Three major types of mammalian models of α -syn aggregation have been utilized for discovery and development of α -syn-targeted therapies: 1) transgenic rodents expressing wild type or mutant α -syn; 2) viral vector-mediated expression of wild type or mutant α -syn; and 3) injection of preformed fibrils (PFFs) of α -syn, Table 2 and the description below briefly highlight the key characteristics of each type of model and their translational utility. Animal models may recapitulate specific features of PD pathology or downstream pathophysiology which can be utilized to assess investigational therapies. In general, investigational therapeutic agents should be tested in multiple animal models to assess efficacy. On the other hand, it is desirable to utilize a single model to establish TE, pharmacodynamic, and PoP endpoints, which can be used to model clinical dosing and define safety margins.

Transgenic rodent models. Several transgenic mouse lines have been created, expressing wild-type or mutant α -syn under the control of a variety of promoters. It is beyond the scope of this article to describe these models in detail, and their different features have been covered extensively by earlier reviews [23, 29–33]. In short, none of the transgenic α -syn mouse models can be viewed as the “perfect” model for development of therapies targeting α -syn. Different transgenic α -syn mouse models mimic various features of synucleinopathies and have specific advantages depending on the scientific questions that are asked. Here we only briefly mention some of the overriding principles that influence pros and cons with existing transgenic α -syn models. The various transgenic mouse lines display different regional patterns and levels of α -syn expression in the brain or periphery. Most of these models display intraneuronal or intragial (MSA-like) α -syn accumulation which

offer the opportunity to establish TE or PoP for the specific forms of α -syn aggregates being targeted. A disadvantage of most transgenic mouse models is that they rarely exhibit significant neurodegeneration in the substantia nigra. As a consequence, PD-like motor dysfunction is typically not a prominent feature. This precludes the ability to correlate TE or PoP to motor dysfunction that is directly relevant to PD. However, non-motor symptoms of PD such as cognitive or olfactory deficits or gastrointestinal symptoms may be taken advantage of, when present in specific models. Furthermore, the level of α -syn expression in transgenic mouse models is frequently much greater than that seen in the human disease. This caveat needs to be considered in the generation of physiological pharmacokinetic-pharmacodynamic modeling or predictive dose-response modeling.

Viral vector-mediated α -syn overexpression Adeno-associated viruses (AAV) are currently the vector of choice to transduce α -syn in the rodent or non-human primate nigrostriatal system. Although AAV models are relatively more labor-intensive and variable compared to transgenic rodent models, the marked α -syn overexpression in nigral dopaminergic neurons following localized AAV delivery can lead to impaired striatal dopamine (DA) release, axonal α -syn pathology in the nigrostriatal pathway, death of nigral dopaminergic neurons with neuroinflammation, and varying degrees of motor deficits [34–38]. Thus, tests of motor function can be used to assess effects of therapies in these models. MSA-like pathology has also been produced in viral transduction models by using specific promoters to drive expression in oligodendrocytes [39, 40]. As with transgenic α -syn models, concerns remain around the relevance of studying α -syn aggregation or downstream sequelae such as neurodegeneration, motor deficits, or neuroinflammation driven by exceptionally high α -syn levels.

Injections of α -syn preformed fibrils or pathological extracts. Over the past six years, several rodent and non-human primate models based on injection of recombinant α -syn PFFs or extracts of pathogenic human brain-derived α -syn into the brain of rodents have been developed [21, 22]. Typically, PFF injections trigger progressive development of intraneuronal α -syn aggregates with associated neurological deficits, the distribution and behavioral correlates of which depend on the brain regions and the type of α -syn injected [21, 22, 41–43]. The

Table 2
Alpha-synucleinopathy-directed therapeutic approaches evaluated in animal models

| Type of Model | Key Characteristics | Exemplary Endpoints to Inform Clinical Translation |
|--|---|---|
| Transgenic rodents expressing wild-type or mutant human α -syn under the control of different promoters | <ul style="list-style-type: none"> • Each transgenic strain displays a specific regional pattern and level of α-syn overexpression • None reproduce the development the full repertoire of neuropathology • α-syn expression in these models is frequently much greater than that seen in the human disease | <ul style="list-style-type: none"> • TE: Therapeutic antibody bound to its targeted α-syn species in the brain and/or accessible biofluids such as CSF and blood compartments • PoP: Reduction in the load of α-syn aggregates |
| Viral vector-mediated over-expression of WT or mutant α -syn in select brain regions | <ul style="list-style-type: none"> • Labor intensive with relatively high inter-animal variability • Possible to achieve marked α-syn overexpression in nigral dopaminergic neurons • Can lead to impaired striatal DA release, axonal α-syn pathology in the striatum, death of nigral dopaminergic neurons with neuroinflammation and varying degrees of motor deficits | <ul style="list-style-type: none"> • TE: Therapeutic antibody bound to its targeted α-syn aggregates or species • PoP: Reduction in the load of α-syn aggregates, dopaminergic axonal degeneration measured by DAT density, neostriatal dopamine release, nigral dopaminergic neuroprotection, neuroinflammation • Functional outcomes: Basal ganglia-mediated behavioral deficits (e.g., hypokinesia) |
| Injection of α -syn PFFs injected into rodent brain or administered systemically (IV, IM) | <ul style="list-style-type: none"> • “Prion-like” propagation of the pathology which follows neural pathways after intracerebral injections • Following intrastratial injection: progressive loss of nigral dopaminergic neurons and development of motor deficits • PFFs injected into the olfactory bulb triggers formation of α-syn aggregates which cause a gradual spread of pathology in multiple brain regions (no reported dopaminergic neuron loss) • Systemic injections of PFFs are also reported to trigger synucleinopathy in the nervous system | <ul style="list-style-type: none"> • TE: Therapeutic antibody bound to its targeted α-syn aggregates or species • Pharmacodynamics: α-syn mRNA or protein levels induced by transcription or translation inhibitors • PoP: Reduction in the propagation of α-syn pathology, dopaminergic neuroprotection • Functional efficacy: Behavioral deficits (e.g., hypokinesia, hyposmia) |

AAV, adeno-associated virus; CSF, cerebrospinal fluid; DA, dopamine; DAT, dopamine transporter; IM, intramuscular; IV, intravenous; mRNA, messenger ribonucleic acid; PFF, preformed fibrils; PoP, proof of principle.

mechanism underlying the PFF models is thought to involve “prion-like”, trans-cellular propagation of the pathology, dependent on endogenous α -syn. Thus, the development of pathology is more rapid when injections are made into transgenic α -syn mice, or animals with viral vector-mediated overexpression of α -syn, than wild-type mice and no pathology develops in α -syn null mice [44–46]. The pathology appears to propagate along connected neuronal pathways in a manner that has been suggested to occur with PD progression [47]. Models where the PFFs are injected into the nigrostriatal system can also show progressive loss of nigral dopaminergic neurons, reduction in striatal DA, and development of motor deficits, allowing for motor testing of functional effects of therapies [44, 45, 48]. More recently, PFFs injected into the olfactory bulb have been shown to produce α -syn aggregation and

neurodegeneration in olfactory structures and a gradual development of specific olfactory deficits akin to hyposmia in prodromal PD [49, 50]. Thus, PFF mouse models appear to be relevant when testing therapies intended to mitigate α -syn pathology in early disease by reducing α -syn expression or increasing clearance of extracellular α -syn. Other variations on the PFF models include injections through peripheral routes, (i.e., intravenous or intramuscular) [26, 51] or directly into the gastrointestinal tract [52–54]. Analogous to the PFF injections into the olfactory bulb, targeting the gastrointestinal tract with PFFs or other forms of α -syn has been suggested to be relevant as a model of prodromal PD. Recent reports, however, suggest that brainstem α -syn pathology triggered by PFF injections into the gut of wild type mice is transient and that there is little or no further propagation to other brain regions [53, 54].

What is the value of experiments in non-human primates?

Due to the relatively low cost and the ease at which genetic modifications can be made, rodent models of synucleinopathy are more abundant and frequently used than non-human primate models. Recently, non-human primate models of synucleinopathy that use AAV vectors to overexpress the protein and injections of PFFs have been generated [55–57]. None of these models have been characterized in great detail yet, and therefore, their place in a drug development program targeting α -syn remains to be determined. In the future, non-human primate models might provide the ability to discover and monitor biomarkers in the cerebrospinal fluid (CSF), which remains a major limitation of rodent models. Additionally, their larger blood volume compartment could allow for longitudinal sampling and assessment of biomarkers and pharmacokinetic parameters.

What are relevant outcome measures and biomarkers for clinical translation?

From a translational perspective, the major utility of the animal models of α -syn aggregation described above is to establish TE, pharmacodynamic responses, PoP, and functional efficacy measures (Table 2). By definition, a TE biomarker has to be specific to the therapeutic agent under investigation, making it hard to offer generalizable recommendations. For example, for therapeutic monoclonal antibodies, measurement of antibody-bound α -syn versus free α -syn levels in the brain, as well as in the CSF and peripheral matrices, can provide useful indices of TE. Furthermore, effects on other markers of α -syn pathology (e.g., pS129-modified α -syn or oligomeric α -syn) could be utilized as pharmacodynamic or PoP outcomes. Similarly, therapies that aim to reduce α -syn transcription *via* antisense mechanisms can rely on direct assessment of α -syn transcripts or protein levels for TE.

Pharmacodynamic biomarkers reflect a change induced by the binding or modulation (activation/inhibition) of the desired target by the therapeutic agent, and as such they provide an additional dimension of drug effect beyond TE. An example of a pharmacodynamic endpoint is the level of the lipid glucosylceramide, the substrate of glucocerebrosidase, for agents that allosterically activate or stabilize this lysosomal enzyme implicated in α -syn clearance.

Neither TE nor pharmacodynamic biomarkers on their own provide insights into the level of TE

or pharmacodynamic responses required to produce clinically relevant efficacy. The integration of a PoP biomarker that measures a key pathogenic event in PD brains helps answer this question. For α -syn targeted therapies, immunohistochemistry for α -syn deposits is an important preclinical PoP endpoint, since Lewy pathology is a defining feature of PD. In animal models, deposits of aggregated α -syn are often assessed by immunohistochemistry using antibodies against pS129 α -syn, since pS129 α -syn also tags Lewy pathology in PD brains [58]. Thus, a reduction in pS129 α -syn staining could be viewed as positive preclinical PoP. Other markers for Lewy pathology (e.g., ubiquitination, p62, measurement or staining of proteinase K-resistant α -syn) and biochemical measures of soluble versus insoluble α -syn aggregates have also been employed to verify or extend PoP findings. The status of development of biomarkers to assess Lewy pathology in humans is detailed in sections 3. Animal models that exhibit α -syn aggregation-associated nigral degeneration offer the opportunity to monitor the effect of the therapeutic agent on the second hallmark pathology of PD *via* stereological cell counts of tyrosine hydroxylase (TH)-immunoreactive neurons.

The dopamine (DA) transporter (DAT), a translational endpoint reflecting integrity of dopaminergic nerve terminals in the striatum, can be measured in the clinic using single-photon emission computed tomography (SPECT) imaging, making assessment of DAT in an animal model, by immunohistochemistry or biochemistry, a particularly relevant preclinical measure. Measuring DA levels or release in the neostriatum also can provide an important functional neurochemical endpoint. Following AAV-mediated expression of α -syn in the substantia nigra, reduced DA release in the striatum (measured using *in vivo* voltammetry) can be seen in some, but not all animal models [59]. Because changes in striatal dopaminergic terminal function are recognized as an indicator of early stage PD, monitoring perturbations in nigrostriatal neuronal function in AAV α -syn-injected rodents is a pertinent readout. Finally, some models show an increase in neuroinflammation, measured by changes in microglia and increases in the mRNA or protein levels of inflammatory cytokines such as IL-1 β , TNF α , and IL-6 [60]. These downstream pathophysiologic markers may be considered for novel therapeutics targeting α -syn.

For behavioral endpoints reflective of basal ganglia dysfunction or olfactory pathology, we refer the readers to several recent reviews (see [18–24]).

Preclinical to human translation

Integrated analysis of animal model data with safety data for translational success

From the discussion above, one can see that it is possible to generate therapeutic mechanism-specific data on TE, pharmacodynamics, PoP, or efficacy in animal models of synucleinopathy. The integration of these outcomes with pharmacokinetic data allow translational pharmacologists to generate physiologically-based pharmacokinetic/pharmacodynamic models to inform dose and dosing regimens for first in human trials. These models can also take into account any dose-limiting safety and toxicity findings observed through incorporation of toxicokinetic data from rigorous preclinical development activities in order to identify therapeutic windows for conducting early stage clinical trials. The pharmacokinetic/pharmacodynamic models should be continuously refined as new clinical pharmacokinetic, safety, tolerability, and biomarker data emerge and inform subsequent clinical decisions.

Considerations for determining target engagement and proof of principle

A recent retrospective review of delayed or denied approval of new medical entities by the Food and Drug Administration (FDA) between 2000 and 2012 indicates that uncertainties related to dose selection is a major factor underlying the failure of drug development programs [61]. As described above, establishing TE in early-phase clinical trials and utilizing pharmacokinetic-TE modeling can minimize the uncertainties in clinical dose selection. In this regard, it is important to take into account the distribution of physiologic and pathologic α -syn in central versus peripheral biofluids and cells. Thus, while A β and tau species targeted by investigational therapies of AD are enriched in the brain and CSF as compared to the blood compartment [62–64], α -syn levels are significantly higher in the plasma and red blood cells than the CSF [65, 66]. This raises the concern that peripheral α -syn could act as a sink for a therapeutic antibody to limit the unbound (free) fraction available for entry into the brain; an issue that can be further accentuated by the generally low brain penetration of monoclonal antibodies (CSF/plasma concentration ratio 0.01–0.04; [e.g. 63]. However, this critical concern may be mitigated by data from recent Phase 1 clinical trials of two distinct anti- α -syn therapeutic antibodies. These data demonstrate the presence of unbound α -syn antibody in the

CSF of trial subjects [67, 68]. Moreover, therapeutic α -syn antibodies may achieve higher brain distribution, and thus TE, by preferential or even selective binding to brain specific conformational epitopes of α -syn assumed to play a more essential role in α -syn pathophysiology.

Another important TE question when designing α -syn therapies is whether the targeted “pathogenic” α -syn species is in the extracellular or intracellular compartment. Consistent with the Braak hypothesis [47], results of PFF-based animal model studies indicate that α -syn aggregates can indeed be transmitted trans-synaptically [18, 19, 21, 22]. Thus, it may in fact be advantageous for antibody therapies to target an extracellular α -syn species, a concept that is supported by the demonstration that anti- α -syn antibodies reduce the density of α -syn aggregates even in animal models that do not rely upon PFF injection.

As stated above, PoP biomarkers, when combined with TE or pharmacodynamic markers are critical data confirming the validity of clinical dose selection. Thus, prior to initiating large, resource-intensive disease-modifying clinical trials, it would be ideal to establish PoP and, if achieved, its dose dependency in a small PD patient study, as exemplified by the evaluations of amyloid plaque removal with A β PET imaging in the Phase 1 studies of the A β monoclonal antibody therapeutics Aducanumab [10] and Gantenerumab [15]. Unfortunately, at the time of writing of this review, imaging methods to monitor Lewy pathology or presumed pathogenic α -syn species in the brain or CSF still represent major gaps in our therapeutic development toolbox (see below: “Proof of Concept Clinical Studies”).

In summary, it is most advisable to select an animal model pertinent to the therapeutic mechanism of choice and integrate TE, pharmacodynamic and PoP biomarkers in order to generate PK-TE/pharmacodynamic/PoP modeling to inform clinical dosing. Data from early stage clinical trials could further refine the modeling and provide a rationale-based approach for PoC and registration trials.

CLINICAL BIOMARKERS OF ALPHA-SYNNUCLEIN PATHOLOGY

With at least five α -syn-targeted therapeutics currently under clinical investigation [69], there is an urgent need for robust and validated biomarkers. Here

we review the current biomarker landscape, with a particular focus on methodologies to measure α -syn in biofluids and peripheral tissues, as well as imaging modalities and mobile technologies. It is important to note that currently available biomarkers have been validated in clinically-defined cohorts of sporadic PD rather than on non-hypothesis-driven (i.e., agnostic to clinical criteria) cohorts of normal and abnormal aging populations. Thus, data supporting the use of these biomarkers represent statistical separations between patients with PD and age-matched controls, but are fraught with substantial overlap [70].

Biochemical assays in biofluids and tissue

Alpha-synuclein immunoassays

Given that accumulation of aggregated α -syn is a hallmark pathology of PD, perhaps the most obvious choice for a TE, PoP, or disease progression biomarker for α -syn-targeted therapies is various conformers and species of α -syn itself (e.g., native monomer, post-translationally-modified monomers, or assemblies such as oligomers or fibrils). In the absence of an α -syn imaging tracer (discussed in greater detail below), measuring α -syn in CSF assumes it to be a measure of brain pathology (and therefore, a PoP biomarker), but this assumption remains to be tested. A number of studies have measured the so-called “total” α -syn in biofluids such as CSF, plasma, and saliva [71–74]. Although these α -syn assays may offer the ability to establish TE for specific drug candidates, several challenges hamper their utility to establish PoP or monitor disease progression in clinical studies. First, since the precise species of α -syn being measured by current commercially available immunoassays of total α -syn remain unknown, it is not possible to demonstrate that the assay reflects a species of α -syn categorized as pathogenic in animal studies. Furthermore, these assays have shown that the levels of α -syn species measured remain unchanged in 12 month follow-up of the Parkinson’s Progression Markers Initiative (PPMI) cohort (Mollenhauer et al., 2018, submitted). Whether the ongoing, longer follow-up of the PPMI cohort or other similar studies might help remediate this issue and enable the use of total α -syn assays to monitor disease progression remains to be seen. Finally, pre-analytical conditions such as CSF collection, processing, and sample handling appear to influence total α -syn measurements [75], indicating careful studies need to be conducted to establish pre-analytical conditions before the

existing assays could be used effectively in clinical trials (see below).

The diagnostic utility of total α -syn has been investigated thoroughly. Numerous studies have demonstrated total α -syn concentrations in the CSF are reduced by an average of 15% at the group level in PD patients compared to healthy controls (reviewed in [76]). However, at the individual level, considerable overlap exists between PD and healthy control subjects which renders the diagnostic use of CSF α -syn problematic [71]. Notably, the reduction in CSF α -syn in PD patients has been demonstrated using a variety of analytically validated immunoassay platforms, including standard chemiluminescent sandwich enzyme-linked immunosorbent assays (ELISA) [71, 72], electrochemiluminescent sandwich immunoassays [73], and bead-based cytometric assays [74]. In an effort to further qualify the total α -syn immunoassays, MJFF sponsored a round robin assessment of four different assay platforms on CSF, whole blood, and saliva samples of 50 PD and 50 healthy control subjects. The four assays selected in this initiative were the Elecsys[®] Total α -Synuclein Prototype Assay (Roche Diagnostics, Penzberg, Germany), the MSD U-PLEX[®] Human α -Synuclein Kit (Meso Scale Discovery, Rockville, Maryland), the BioLegend α -Synuclein Immunoassay (BioLegend, San Diego, CA), and the ADx α -Synuclein Immunoassay (ADx Neurosciences, Gent, Belgium). Blinded analyses of the biospecimens were conducted by both the originating laboratory that developed the assay and satellite laboratories equipped to run the assay. The mean intra-platform variance component ranged from 8.12% to 9.81%, an acceptable range. Importantly, the four assays correlated well to each other, even though the absolute concentrations of α -syn measured in the same samples were different (Mollenhauer et al., 2018, in revision). The results of this study highlight the need for common reference materials and methods to control for inter-assay variability. An investigation of peri- and pre-analytical variables affecting assay performance was also undertaken as part of this work, and recommendations from MJFF’s Investigating Synuclein Consortium investigators for ways to reduce α -syn assay variability through controlled and consistent sample handling methods have been published [75].

Immunoassays to detect specific, pathologically-relevant α -syn species have also been generated using isoform-specific antibodies. For example, an increase in pS129 α -syn levels has been reported

in the CSF of PD subjects compared to healthy controls, although it is unclear whether this species changes over the course of the disease and correlates with disease severity [72, 77]. Likewise, higher levels of oligomeric α -syn have been reported in PD CSF compared to healthy controls, and they also appear to increase over time [72]. These “modified” α -syn assays have mainly been developed in academic research laboratories, have not been cross-validated by multiple groups in blinded samples, and are not generally available for widespread use by the research community. For this reason, MJFF has undertaken another initiative to analytically validate the pS129 and oligomeric α -syn assays through a broader round robin consortium known as LEAPS (Linked Efforts to Accelerate Parkinson’s Solutions). Research is also in progress to identify novel potentially pathological species of α -syn for which assays could be developed in the future (e.g., truncated, oxidized, phosphorylated at sites other than S129). In addition, biochemical properties of α -syn, such as its ability to bind to lipids, have been investigated as potential differentiating biomarkers, and independent cross-validation and replication efforts are currently underway [78].

Seeding and turnover assays

The immunoassays described above are dependent on the quality and availability of antibodies for specific detection of the relevant form(s) of α -syn. New antibody-independent assay approaches for detection of α -syn aggregates via repeated cycles of seeding, amplification, and break down, similar to DNA amplification by polymerase chain reaction, appear promising. Two variations of this seeding assay technology, protein misfolding cyclic amplification assay (PMCA) and real-time quaking-induced conversion (RT-QuIC), have demonstrated high sensitivity and selectivity in differentiating PD from healthy control subject CSF in initial studies [79, 80]. Efforts are also under way to measure α -syn turnover through stable isotope labeling kinetic (SILK) assay development. This technique measures incorporation of a stable isotope amino acid tracer into newly synthesized α -syn, measured by mass spectrometry in CSF collected from living subjects [81].

Immunohistochemical analysis in the periphery

Given the hypothesis that peripheral α -syn pathology may develop prior to that in the CNS, and in the absence of an α -syn imaging tracer, considerable effort has been devoted to measuring α -syn

deposition in peripheral tissues. Several studies (reviewed in [82]) report inconsistent results in colon and skin biopsies, possibly a result of the many permutations in collection and processing protocols utilized or variable distribution of α -syn in the tissues examined. The submandibular salivary gland has emerged as a potentially sensitive and specific peripheral biomarker differentiating PD and healthy control subjects [83], although transfer of this technique and replication by multiple groups, as well as assessment of the feasibility of collection in a multi-site trial, is pending. The ongoing Systemic Synuclein Sampling Study (S4) is designed to evaluate α -syn in three peripheral tissues, skin, colon and submandibular salivary glands, and correlate it with biofluids levels (CSF, saliva, and blood) collected from the same subject [84].

Physiological biomarkers

Physiological biomarkers provide additional opportunities for segregating PD and healthy control subjects and may be relevant as phenoconversion markers of prodromal PD (reviewed in [85]). Functional, non-invasive techniques such as heart rate variability, electroencephalography, electromyography, optical coherence tomography, and skin conductance have shown potential utility as relevant functional biomarkers of α -syn pathology. Future replication through natural history studies of large cohorts of PD as well as non-PD neurodegenerative Parkinsonisms may elucidate whether a combinatory physiological “PD signature” will emerge, sufficiently distinct from PD-like disorders. There is also ongoing development of new physiological biomarker platforms, including pilomotor reflex [86], breath volatile organic compounds [87], eye tracking [88] and corneal confocal microscopy [89], facial expressivity [90], and acoustic speech [91]. Correlation of all of these physiological measures to α -syn pathology will be an important determinant of potential integration of these measures into clinical trials. Wearable and interactive devices (see 3.3) may also be considered a type of physiological biomarker.

Imaging

While other biomarkers are evolving, the most robust markers for assessing PD progression (and presumably, the effects of disease-modifying interventions) are based on neuroimaging and, for now, particularly those that assess dopaminergic neuron function. Dopaminergic imaging, discussed in

further detail below, may not be optimal for a variety of reasons. Here we consider other possible imaging approaches to studying PD progression (Table 3).

Structural imaging

Standard diagnostic MRI is of limited use for confirming a PD clinical diagnosis but may be helpful to exclude atypical forms of Parkinsonism such as MSA or progressive supranuclear palsy (PSP) [92]. This is an important consideration, as inclusion of patients with atypical disease could dilute the results of any interventional study. More sophisticated approaches to structural MRI may prove informative. Striatal volume declines over time, particularly in the early stages of PD [93], while cortical thinning and hippocampal atrophy are associated with cognitive decline [94]. The pattern of atrophy in PD may progress according to a defined network of connectivity to the substantia nigra, compatible with propagation along anatomical pathways [95]. Susceptibility weighted or T2* imaging provide insight into brain levels of iron. Nigral iron signal increases in association with clinical measures of disease severity in PD [96, 97], and this may accordingly represent a relatively inexpensive and widely available approach to quantify disease state. T2* weighted imaging at high field strengths detects a dorsal nigral hyperintensity (DNH) deemed analogous to the calbindin-negative nigrosome 1 [98]. This DNH is lost both in PD and in a majority of subjects with REM sleep behavior disorder (RBD) [99], is preserved in non-degenerative forms of Parkinsonism, and predicts loss of DAT binding [100].

More experimental MRI techniques include neuromelanin imaging and tract-tracing techniques such as diffusion tensor imaging (DTI). Reduced neuromelanin signal has been reported in PD [101], may decline as disease progresses [102, 103], and may indeed correlate in some hands (but not others, [104]) with striatal DAT binding [105], but this is currently not an approach in wide use. DTI examines the movement of protons and is widely used to assess fiber tracts. This technique has revealed reduced fractional anisotropy (FA) in the posterior substantia nigra in PD [106], and changes in FA in the nigrostriatal tract appear to correlate with clinical measures of disease severity [107]. Furthermore, reduced nigral FA correlates with increased free water in PD, and, when assessed using a bitensor model, increases with disease progression [108].

Functional imaging

Resting state. Resting state functional MRI (rs-fMRI) has revealed reductions in connectivity of the pre- and post-central gyri, occipital cortex, and cuneus in PD that progresses over the course of illness and correlates with cognitive decline [109]. The potential validity of using a change in patterns of rs-fMRI connectivity to assess disease progression is highlighted by a study demonstrating a correlation between reduced sensorimotor connectivity and reduced CSF levels of α -syn [110]. However, it should be noted that all rs-fMRI studies to date have been small and single-site. Feasibility in multicenter trials is still a concern.

Task-related activity. PD is typically associated with reduced activation of the supplementary motor cortex (SMA) during performance of a simple motor sequence, and activation can be restored by symptomatic medication such as apomorphine [111]. While this may seem a relatively non-specific outcome measure, it may be important to demonstrate the functional relevance of improvement in other markers. Increased dopaminergic activity was seen within six months after fetal mesencephalic transplantation for PD, but maximal clinical improvement did not occur until later, associated with increased activation of the SMA, presumably reflecting a gradual and protracted integration of the graft into the cortico-striatal-pallidothalamo-cortical loop [112]. Cognitive activation studies may also provide insight into disease state, but interpretation is challenging. Performance of an executive task should be associated with suppression of activity in the Default Mode Network (DMN). In patients with PD, there is failure of suppression in the DMN [113], but the relationship to disease progression and to symptomatic therapy is unknown. Changes in activation patterns can be seen even in presymptomatic carriers of *LRRK2* mutations during performance of a motor imagery task or while performing the Stroop interference task [114, 115]. It is unclear how these alterations change with disease progression, but they are thought to represent compensatory shifts and highlight the challenges in interpreting such data.

The pattern of cerebral glucose metabolism, as measured by fluorodeoxyglucose positron emission tomography (FDG-PET) imaging, is altered in PD in a characteristic fashion, i.e., relatively increased metabolism in the basal ganglia, thalamus, pons and cerebellum, with concomitant relative reductions of metabolism in the premotor and parietal cortices

Table 3
Potential imaging biomarkers for Parkinson's disease

| | Modality | Indication | Caveats | Other comments |
|--------------------|--|--|---|---|
| Structural MRI | High-resolution T1, T2*/GRE, FLAIR | Exclusion of atypical Parkinson syndromes | Differences may be subtle, limiting the utility | – |
| | Subcortical volumetry | Exclude atypical Parkinson syndromes | – | – |
| | Cortical thickness, hippocampal volume | Thinning/atrophy associated with cognitive decline | Patterns likely to be heterogeneous depending on clinical phenotype | Pattern of atrophy linked to nigral connectivity and may progress according to a defined network of propagation |
| | Neuromelanin | Assessment of dopaminergic and noradrenergic cell bodies | Small volume of aminergic nuclei makes reliable quantitation difficult | Need data from longitudinal studies to understand potential as progression marker |
| | Diffusion weighted | Free water | Initial studies not independently reproduced | Nigral free water increases with disease progression but unclear how robust after early time points |
| | Susceptibility weighted or T2* | Iron sensitive | Increase may not be robust enough to be useful | Increased iron with disease progression |
| Functional Imaging | Resting state fMRI | Connectivity of pre- and post-central gyri, occipital cortex and cuneus | Difficult to control quality of images in multicenter studies | Altered connectivity correlates with cognitive decline and with CSF α -synuclein |
| | FDG-PET | Parkinson Disease Related Pattern | PDRP is suppressed by symptomatic therapies; requires sophisticated statistical analysis; some controversy regarding global normalization (As above) | Diagnostic value and changes with disease progression |
| | | Parkinson Disease Cognitive Pattern | | PDCP is linked to cognitive decline, a greater source of disability in advanced disease |
| | Task-related activity | Motor activation | Difficult to standardize across centers. Primary utility is for research studies rather than clinical trials | Provides evidence of functional integration |
| Cognitive tasks | | Difficult to standardize across centers. Primary utility is for research studies rather than clinical trials | Alterations in prodromal disease reflect compensation; unclear role in studying disease progression | |
| Molecular Imaging | DA systems: DAT SPECT or PET | Assessment of dopamine nerve terminal integrity, primarily in striatum | DAT may be subject to pharmacological and compensatory regulation | Widely available. Different tracers have varying selectivity and kinetic properties |
| | DA systems: VMAT2 binding | More closely approximates monoamine nerve terminal density | Less widely available than DaT, not specific for DA | May track disease progression but additional data are needed. |
| | DA systems: F-dopa uptake | Assessment of presynaptic dopamine nerve terminals and dopamine synthesis rates | Not widely available; decarboxylase is subject to regulation and not specific to dopaminergic neurons | Historical 'gold standard'. Prolonged scans can be used to assess DA turnover |
| | Non-DA systems | Cholinergic (cholinesterase, VAcHT); serotonergic (SERT) | Cholinesterase activity cannot be quantitated in striatum, but VAcHT can | Cholinergic dysfunction relates to several aspects of disability, especially cognition |

(Continued)

Table 3
(Continued)

| | Modality | Indication | Caveats | Other comments |
|----------------|---------------|--|---|---|
| PoP Biomarkers | α -syn | Quantification of alpha-synuclein pathology in brain | No specific tracers to date; intra-cellular localization and limited brain density suggests signal will be limited; possibility of peripheral consumption | 'Holy grail' of synucleinopathy imaging biomarker |
| | TSPO | Microglial activation | Classical ligand has low signal; binding of newer agents varies according to polymorphism; quantitation difficult | Unclear how microglial activation progresses with disease and whether it is harmful or protective |

CSF, cerebrospinal fluid; DAT, dopamine transporter; fMRI, functional magnetic resonance imaging; F-dopa, fluorodopa; FDG, fludeoxyglucose; MRI, magnetic resonance imaging; NM, neuromelanin; PD, Parkinson's disease; PDCP, Parkinson's disease cognitive pattern; PDRP, Parkinson's disease related pattern; PET, positron emission tomography; PoP, proof of principle; SERT, serotonin transporter; SPECT, single-photon emission computed tomography; TSPO, translocator protein; VAcHT, vesicular acetylcholine transporter; VMAT2, vesicular monoamine transporter 2.

(the PD-related pattern, PDRP). PDRP expression increases with disease progression and is inversely correlated to DAT binding in the putamen [116]. However, the utility of the PDRP as an imaging biomarker of disease progression is unclear because the pattern is suppressed by symptomatic therapies including levodopa and deep brain stimulation [117]. While the PDRP correlates with motor dysfunction in PD, trials of α -syn-based therapies should also take into consideration the cognitive decline that represents a significant source of disability for a substantial number of patients. The PDCP (PD cognitive pattern) is a distinct network of altered metabolism that has been found to correlate with cognitive change and with DAT binding in the caudate nucleus rather than the putamen [118]. PDRP and PDCP may be measured in the same set of images. FDG-PET may serve to differentiate PD from other akinetic rigid syndromes such as MSA or PSP [119]. This potential diagnostic utility may be highly relevant to the conduct of trials of disease-modifying therapies, as these conditions can be difficult to diagnose in early stages, are not readily differentiated by dopaminergic tracer studies, and inclusion of such patients may reduce the chances of detecting therapeutic benefit.

Molecular imaging

Molecular imaging of the nigrostriatal dopaminergic system is still likely the most widely accepted biomarker to assess PD progression and, by extension, the impact of potential disease-modifying therapies. The most extensively used approach is some form of imaging presynaptic dopaminergic neuron integrity. While this clearly addresses the most

obvious deficit in PD, it is probably not the only outcome of interest, as progression of PD and major sources of disability are associated with changes in several non-dopaminergic neurotransmitter systems, and neurochemical studies provide only one measure of the underlying pathology.

Dopaminergic measures. The most widely used approaches utilize PET or SPECT to assess expression of the membrane DAT, the vesicular monoamine transporter type 2 (VMAT2), or activity of L-aromatic amino acid decarboxylase (L-AAAD). All three approaches provide similar, although not identical, information on the integrity of dopaminergic nerve terminals in PD. All can demonstrate the characteristic asymmetric reduction of tracer uptake in PD, with a rostro-caudal gradient in which the posterior putamen is maximally affected. All approaches have, to varying degrees, demonstrated a correlation between tracer uptake and nigral dopaminergic cell counts [120–124]. Furthermore, the progression of abnormalities using any of the three approaches [125] is remarkably similar to the pattern of striatal TH and nigral dopaminergic neuron loss demonstrated in post-mortem PD studies [126], with an exponential pattern of decline that reaches an asymptote at four-to-five years into the disease. Nonetheless, considerable caution is required in the application of these approaches to study disease modification. Although the practical significance is uncertain, both L-AAAD activity and DAT are subject to pharmacological and compensatory regulation, particularly evident in early PD stages [127, 128]. While there is a general correlation between clinical measures

of PD severity and imaging markers, it has been increasingly difficult to demonstrate a correlation between changes over time in the two parameters, regardless of which tracer is used (see [129]). Furthermore, there are several examples of discordance between imaging and clinical measures of improvement, particularly related to trials of putative disease modification [130–133]. Thus, while dopaminergic neuron imaging undoubtedly serves as a useful adjunctive biomarker to assess the effects of potential disease-modifying therapies, findings must be interpreted in the light of other available data, and it is unlikely to ever become a surrogate endpoint.

An interesting molecular imaging technique involves uptake of 6-¹⁸F-fluoro-L-dopa (F-dopa) which reflects not only the activity of L-AAAD (a marker for the health of dopaminergic nerve terminals), but also trapping of 6-¹⁸F-dopamine (F-DA) in synaptic vesicles, prolonged scans with F-dopa can therefore be used to assess DA turnover [134]. However, F-dopa itself is only available at a few centers worldwide, and turnover studies are technically challenging, requiring scan times of up to four hours, arterial sampling, and high-performance liquid chromatography for metabolite analysis.

Non-dopaminergic systems. Significant disability in PD is attributable to non-motor complications, many of which presumably reflect changes in non-DA systems. Serotonergic neurons of the raphe are affected in PD, but binding of the serotonin transporter (SERT) ligand ¹¹C-3-Amino-4-(2-dimethylaminomethyl-phenylsulfuryl)-benzotrile (DASB) is only minimally reduced, with no clear correlation to disease severity. In fact, increased ¹¹C-DASB binding is seen in PD patients with depression [135, 136], and relative preservation of ¹¹C-DASB binding in the putamen is associated with levodopa-induced dyskinesias [137, 138]. DASB binding is also increased in multiple brain regions prior to clinical evidence of disease onset in subjects with *LRRK2* mutations [139]. It is therefore unlikely SERT binding will be useful as a marker of treatment efficacy, although a dramatic increase in SERT binding might be associated with treatment-related complications.

Cholinergic dysfunction is common in PD and is associated with cognitive impairment, postural instability, olfactory impairment, and RBD [140–143]. Cholinergic dysfunction with these disabling non-motor features suggests that it may be a meaningful measure of disease activity, particularly with respect

to therapies targeting α -syn. To date, most studies of cholinergic function in PD have been conducted using PET with tracers for acetylcholinesterase [144]. This provides a less direct measure of cholinergic neuronal integrity, and recent development of tracers for the vesicular acetylcholine transporter [145] may offer a more reliable measure and permit easier quantitation, particularly in the striatum.

Both sympathetic and parasympathetic nervous systems are affected in PD, albeit to somewhat varying degrees (see [146] for review). The unequivocal presence of cardiac sympathetic denervation as detected by ¹²³I-metalodobenzylguanidine (MIBG) or ¹¹C-hydroxyephedrine (HED) SPECT, or 6-¹⁸F-fluorodopamine PET, might help differentiate between PD and other atypical parkinsonian conditions such as MSA or PSP, in which cardiac sympathetic denervation is not thought to be present, but even this is controversial. There are similarly recent reports of gastrointestinal cholinergic denervation in early PD, which would not be expected in the atypical Parkinsonisms. However, in both cases, the degree of denervation is highly variable, and there is insufficient evidence of a clear relationship to disease progression that would justify the use of such approaches to monitor either disease progression or the effects of a disease modifying therapy. Furthermore, these peripheral abnormalities are present long before motor manifestations are expressed, as is evident from studies conducted in idiopathic RBD [147], thus suggesting the likelihood of a floor effect in many patients.

Pathology or pathophysiology markers for proof of principle and patient enrichment

The most useful imaging biomarker to establish or confirm the impact of α -syn-based therapies would be a probe for α -syn itself. Thus, a PET ligand to measure α -syn deposition in the brain is highly coveted as a PoP biomarker, analogous to the utility of A β PET tracers in clinical trials of amyloid-targeted therapies [15, 148, 149]. If an α -syn PET tracer would become available for clinical use, there are some important considerations for using it as a PoP biomarker for drug development. Most importantly, one has to take the drug's mechanism of action into account; i.e., would the PET signal need to detect effects of the drug on progressive aggregation and propagation of misfolded α -syn, or is a decrease of already established α -syn pathology expected (clearance effect)? In either case, quantitative methods will have to be developed, with the identification of relevant regions

of interest and reference region. Once the directionality of the effect and quantitative methods have been established, a pre-set target for the desired effect size has to be defined. Each factor discussed above could have a major influence on the sample size and treatment duration, and consequently the ability to obtain a robust PoP based on a PET readout. Additionally, a meaningful go-decision based on α -syn PET imaging, would also have to assume a relationship between the treatment-induced directionality and extent of the effect on the PET signal with a clinical benefits. This critical aspect is well illustrated by the use of amyloid PET imaging in AD programs. Thus, treatment with BACE inhibitor, that reduced CSF A β peptide levels [150] and prevented further increase in amyloid deposition, as demonstrated by PET imaging, did not provide any clinical benefit [16]. On the other hand, there are findings suggesting that treatment with an anti-A β monoclonal antibody leads to amyloid clearance (reduction from baseline) accompanied with a potential delay in clinical disease progression [10]. What further complicates the possibility translate PoP findings to potential clinical benefit is that the stage of disease at treatment may impact the outcome, with stronger effects presumably attainable at earlier disease stages [151]. Beyond the PoP utility of an α -syn PET ligand, it could provide a critical tool for patient enrichment via assessment of α -syn pathology load as well as to monitor the progression of PD pathology in natural history and therapeutic intervention studies.

The discovery and validation of an α -syn-targeted PET ligand has proved elusive for several reasons [152]: 1) while a number of PET ligands have been shown to bind α -syn, these compounds bind to proteins that take on a β -pleated conformation, thus selectivity is generally poor (e.g., BF227 [153] and SIL23 [154] bind to A β and tau, although BF227 did not bind in A β -negative DLB); 2) unlike A β , α -syn is predominantly intracellular and expressed in small quantities, thus adequate cellular penetration and signal-to-noise ratio will be difficult to accomplish; 3) greater selectivity might be afforded by radiolabeled antibodies, but passage across the blood-brain barrier will be quite limited; and 4) kinetics may be challenging, as suggested by the time required (several days) for a molecule to achieve equilibrium [155]. As α -syn is highly expressed in tissues other than the brain, the question arises as to whether a PET tracer would achieve a significant signal in the brain or whether the signal would be consumed peripherally. The fact that one can detect presumed off-label binding of the tau ligand PBB3 to α -syn

in brain (at least in high concentrations) [156] suggests this may not be an issue. Other ^{11}C and ^{18}F tracers that have demonstrated brain penetrance with rapid washout kinetics are in development [157], but selectivity for α -syn and utility in transgenic animal models or humans with synucleinopathies have yet to be demonstrated *in vivo*.

As noted above, the emergence of cognitive impairment is a major clinical aspect of PD and could potentially be an important measure of a clinically meaningful impact of disease-modifying therapies targeting α -syn. Several studies have used PET to determine the presence of abnormal A β deposition, but the prevalence is overall too low [158] to make A β PET a useful biomarker for this purpose.

α -syn interacts with phosphorylated tau [159], and both proteins appear to be co-deposited (along with A β) in PD with cognitive impairment [160], raising the possibility that tau imaging might provide indirect insights into α -syn deposition. Several PET ligands for misfolded tau have been recently been developed. However, the most widely used PET tau tracer, ^{18}F -AV 1451, only binds to a limited extent in discrete cerebral cortex regions in patients with cognitive dysfunction associated with PD or DLB [161]. Emerging evidence suggests that another preferential tau ligand, ^{11}C -PBB3, binds to α -syn, but likely only where there is a high concentration of aggregates, such as in MSA or individuals with *SNCA* multiplication [156, 162].

PD and other neurodegenerative disorders are associated with microglial activation [163, 164]. The 'peripheral benzodiazepine receptor' (now referred to as Translocator Protein, TSPO) ligand ^{11}C -PK 11195 has been used to study PD, with variable results. One study showed localized binding in the substantia nigra that correlated with disease severity [165], whereas another found more widespread inflammation that did not correlate with clinical severity [166]. ^{11}C -PK 11195 is a difficult ligand to work with, as the signal-to-noise ratio is low, and the lack of a clear reference region makes modeling challenging. Several newer TSPO ligands have been developed, but while the signal is better compared to ^{11}C -PK 11195, the binding of these agents varies, subject to genetic polymorphism [167]. Thus, interpretation requires polymorphism testing, and low affinity binders, representing $\leq 10\%$ of the population, cannot be studied. Only limited data are available on TSPO binding in PD using these later generation ligands, and it is unclear how useful they will be [168]. Furthermore, the relationship between microglial activation and disease progression is unclear.

Application of molecular imaging approaches

There is no single imaging measure that can reliably provide a measure of treatment effectiveness. Nonetheless, molecular imaging may prove useful in PoC studies. The ability to demonstrate blood-brain barrier passage and TE would be an important first step in deciding whether or not to proceed with a potential therapy and in the interpretation of failed therapeutic efficacy.

As noted above, measures of nigrostriatal dopaminergic system integrity will inevitably be regarded as necessary to exclude non-DA-dependent Parkinsonism (SWEDD, scans without evidence of dopaminergic deficit), and they would likely constitute a secondary outcome measure in PD trials. Indeed, DAT SPECT has recently been approved by the European Medicines Agency as an enrichment marker for inclusion in clinical trials (draft qualification opinion - [169]), and the FDA has also issued a letter of support for this use [170]. However, although in one study, density of DAT in striatum did correlate inversely with density of α -syn staining, 1) the relationship between change in a dopaminergic marker and change in clinical function is inconsistent [129]; 2) meaningful disability associated with PD progression arises from non-dopaminergic mechanisms; 3) the treatment may have a pharmacological effect on the dopaminergic marker, independent of changes in disease severity; and 4) it may take considerable time and a large patient cohort to demonstrate a convincing effect on the DA-related marker. If robust imaging measures of disease pathology were available, there is still no guarantee that an effect of the treatment on the imaging marker would correspond to an impact on disease progression.

Digital and wearable technologies

Mobile technology-derived measures in human anti-alpha-synuclein trials

Assessment of human behavior using technology placed on the surface or inside the body of a person, or in the immediate environment, has been increasingly recognized as a powerful source of data that may enhance diagnosis and management in medicine (Fig. 2). “Technology” here means detection systems for biological, chemical, and other processes. *Mobile* technology is worn on the body (so-called “wearables”), and its development has been accelerated, fueled by collaborations with the health and fitness sector industry. This technology allows for the detection of subtle changes in human activity

and behavior, changes that may escape detection by traditional measurements, such as diaries, questionnaires, or even in-clinic assessments by trained clinicians. Mobile technology-derived measures are thus attractive options to include as outcome measures in trials of symptomatic and disease-modulating compounds. Due to the nature of PD (associated with predominantly motor symptoms including mobility limitations, falls, and sleep problems; exhibiting a chronic and progressive course), this disease is an interesting “target disease” for the assessment of mobile technology-derived measures. This interest is reflected by many observational studies recently published (for a review, see [171]).

Potential of mobile technology-derived measures

Objective data from mobile technologies can quantify single and recurrent events of interest, extended continuously over a timeframe that captures their frequency in the background of expected fluctuations, or intermittently, offering the opportunity to reliably include a virtually infinite number of highly accurate “visits” and data points. Moreover, measures obtained from mobile technologies can support supervised assessments in the clinic and laboratory, where outcome measures mainly reflect *functional capacity* (“How well can you perform?”) and easily can be used under unsupervised conditions in ecologically natural environments, where the outcome measures reflect *functional activity* (“How do you regularly perform?”) [172, 173].

The high accuracy and inter-event reliability in the measurement of endpoints of interest by mobile technologies promises to increase the signal-to-noise ratio, thereby reducing the sample size required by clinical trials [174]). It could also lower trial costs by shortening the duration and lowering the burden on patients whose efforts no longer require answering diaries or questionnaires [175, 176]. Furthermore, use of mobile and interactive technologies may reduce the need for in-clinic assessments in clinical trials, further reducing burden, complexity and expense. A condensed overview of short-, medium- and long-term efficacy, efficiency, and patient centricity benefits expected from mobile technology-based measures, integrated in a battery of assessments built into clinical trials are available [177].

Why is it so difficult to develop mobile technology-derived measures?

The integration of mobile technology in clinical trials has been difficult for a number of reasons.

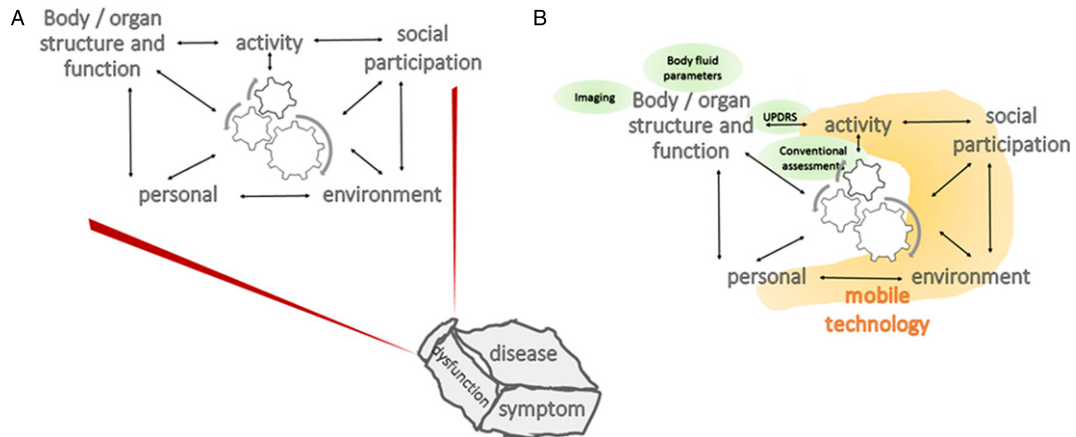


Fig. 2. Adapted Illustration of the International Classification of Functioning, Disability and Health Model of the World Health Organization. A) The description (and treatment) of a disease, dysfunction or symptom require(s) consideration of multiple domains of an individual (more specifically, body/organ structure and function, activity, social participation, as well as personal and environmental factors), and the corresponding interactions across domains. B) Traditional outcome measures, such as clinical scales, body fluid parameters and imaging (green circles) document variables about the body/organ (here: brain) structure and function domain and to some extent about the activity domain. Mobile technology (orange) has the potential to collect information over most of the ICF domains. ICF, International Classification of Functioning, Disability, and Health; UPDRS, Unified Parkinson's Disease Rating Scale.

First, there is a lack of studies investigating mobile technology-derived measures in PD that convincingly describe endpoints of interest. Importantly, as a field we lack consensus defining the optimal concepts of interest and contexts of use for technologies in PD clinical trials. This is due in large part to the fact that the maturity of mobile technology in terms of medical use is low. A recent review found that only 5 percent of about 850 original studies applying technology-derived measures in PD used systems that were close to or already positioned on the market [171]. Furthermore, only a small proportion of studies provide qualitatively acceptable data in terms of cohort and algorithm description, sensitivity to change, specificity, accuracy, and predictive value of investigated measures [171]. Finally, technological development does not follow clinical decision-making, and often the opposite is true. Researchers often focus on seemingly easy-to-detect symptoms or signs with questionable clinical meaningfulness or allow the technology at hand to dictate the questions being asked of subjects.

The multidimensional and protean nature of PD's clinical manifestations further complicates the selection of concepts of interest and contexts of use for the application of technologies; distinct features of PD occur at different time points and deteriorate with different slopes of progression during distinct PD phases. As an example, fine motor movement of

the upper extremities may be a useful progression parameter in prodromal and early, but not late PD phases [178–180]. Moreover, our “pre-technology era” clinical view may hinder us from discovering the clinical relevance and usefulness of PD-related features particularly sensitive to technology-based assessment strategies: candidate measures could be loss or preservation of variability (e.g., of movement “episodes” and activity patterns during the day and during the night); loss or preservation of similarity of comparable movements; phenomena associated with multitasking-related deficits, and a range of non-motor behaviors (e.g., heart rate variability, sleep architecture, etc.). Fourth, we have not yet solved retention issues regarding the use of mobile technology over long periods, especially when they require active participation of the study subjects (e.g., entering data). For example, only two percent of those who had downloaded the mPower app during the first six months of availability used it more than five times [181]. Other studies have reported similarly poor long-term adherence to technology requiring active participation. Thus, such assessments not only suffer from low retention but also from a potential selection bias, as the study participants adopting and using mobile technologies may not be representative of a population of interest. Such lack of adherence could create issues for data completeness and integrity in clinical studies. Fifth, access (see below) and

regulatory issues make it difficult for investigators to integrate these new tools. Raw data is often not freely accessible, and is only interpretable by using company-owned algorithms, and there are often separate proprietary platforms required for devices from different companies, preventing the time-locked integration of their data. Importantly, the field currently lacks data and metadata standards that would allow sharing and common interpretability of device data. Finally, legal (privacy) and regulatory barriers exist and often differ across countries.

In spite of these challenges, the potential of mobile technology-based measures as exploratory endpoints in anti- α -syn trials is enormous, specifically if we consider the following recommendations.

Recommendations for the integration of mobile technology-derived measures in human anti-alpha-synuclein trials

The International Parkinson and Movement Disorders Society Task Force on Technology [176] and the Clinical Trials Transformation Initiative recently published general recommendations for the development of mobile technology-derived measures and for the implementation of mobile technology in PD clinical trials [177]. There is broad consensus that every ongoing clinical PD trial should integrate mobile technology-derived measures, initially on an exploratory level and targeting endpoints previously validated in clearly defined cohorts.

- There is no convincing evidence currently available that TE of pathological α -syn can lead to improvements in *specific* PD symptoms; as such, mobile technology should gather data about a wide range of symptoms, using different modalities (e.g., combinations of accelerometers, gyroscopes, magnetometers, GPS, heart rate) and body surfaces instead of assessing single symptoms and applying single sensors for data collection. Shorter assessment periods (e.g., days rather than weeks of assessment) may compensate for the higher effort on the part of the patients.
- Data obtained from the habitual environment likely have a higher potential to add novel information to a battery of measurements than mobile technology-derived data collected in the clinic and laboratory. Note that traditional assessments mostly examine *functional capacity*, which do not necessarily reflect behaviors in habitual environments, under unobserved

conditions. We recommend performing combined assessments covering functional *capacity* and functional *activity*.

- Data collection approaches that do not require (much) active engagement of the study participants during the active data collection phase should mitigate the current rate of attrition. Translation of these data into understandable information to the technology wearers during or after the active data collection phase may enhance adherence [182].
- If the aim of an α -syn targeted trial is to reduce the progression of progressively worsening symptoms rather than improving potentially non-progressive symptoms, there should be enough evidence that the target symptoms exist in an as large as possible proportion of study participants, have a high chance to be in a “progressive phase” of the disease, and can be measured by technology without the risk of ceiling or floor effects. Interesting candidate features in prodromal and early PD are fine motor movements of the hands and arms and axial symptoms, such as gait and postural instability, at later stages.
- Mobile technology collecting and providing free access to raw data (i.e., the sensor signal) has the advantage that the PIs can contribute to independent data analysis approaches as soon as new algorithms and statistical strategies are available. Moreover, PIs may benefit from open-access platforms for mobile technology that may become available in the near future.

PROOF OF CONCEPT CLINICAL STUDIES

As a key mediator of PD pathogenesis, therapeutic modulation of α -syn-associated pathogenic mechanisms are anticipated to produce clinical efficacy reflective of slowing of disease progression (i.e., disease-modification). The scope of the effort required to unequivocally demonstrate slowing of disease progression, with an appropriate safety and tolerability profile, will require the design, conduct, interpretation, and regulatory review of data generated from multiple clinical trials. The breadth of such a development program, which will be highly dependent upon the size of the beneficial clinical effect observed, is likely to require hundreds of biomarker-enriched (and potentially thousands) of

patients studied over relatively long time periods (months to years). Such efforts, as exemplified in recent AD therapeutic development efforts, will be resource intensive, the bulk of which expended at the latter stages of clinical evaluation (i.e., pivotal or registration studies). Therefore, the development of an α -syn-based therapeutic should progress in a step-wise manner in which advancement decisions are guided by the strength of the aggregate preclinical, non-clinical and clinical data.

Previous sections in this white paper have covered translational approaches, tools and measures currently available to assess the therapeutic potential of a given α -syn-directed drug candidate. It is imperative to continue to characterize and refine these tools and outcomes in a translational manner to optimally design initial clinical studies. The focus of this section is to discuss key early clinical development objectives and clinical trial elements for α -syn-based therapeutics, with specific emphasis on advancement to PoP and PoC. The challenges and issues faced in latter stages of clinical development (pivotal/registration trials) of an α -syn-based therapeutic (or any other potentially disease-modifying therapy for a neurodegenerative disease) (recently reviewed in [69]) will not be addressed herein.

What defines proof of principle and proof of concept for alpha-synuclein-based therapeutics?

PoP and PoC relate to the level of confidence one assigns to an available dataset at key decision points during development. In the absence of a uniformly accepted definition, the present discussion will emphasize that PoP is defined by data generated in humans demonstrating that a potential therapeutic can be administered safely at dosages (or, more accurately from a translational perspective, exposures) that lead to a measurable change in a pathological or pathophysiological endpoint assessed by physiological, biochemical, or imaging biomarker. Similarly, PoC is defined by data generated in humans that a potential therapeutic favorably impacts a clinical outcome, or a validated biomarker that may reliably predict clinical efficacy when studied in a larger, perhaps more diverse, patient population. For instance, lowering α -syn levels in a central or peripheral tissue compartment after short-term exposure (days to weeks) without seeing a beneficial clinical outcome may constitute PoP. On the other hand, demonstrating slowing of motoric decline after longer-term (likely months/years) exposure, preferably with a reduction

in brain α -syn load via a PET tracer, may constitute PoC. When considered in these broad terms, the goals of any early clinical evaluation of an α -syn-based therapeutic should be to initially establish robust PoP as a foundation to support the latter demonstration of clinical PoC. As we have argued earlier, it is difficult to envision achievement of clinical PoC for an α -syn-based intervention without first demonstrating PoP.

Symptomatic effects versus disease modification

Clinical assessments for symptomatic outcomes should be included in all initial, short-duration treatment studies as it is a confounding factor that influences the design and interpretation of later, larger pivotal studies seeking to demonstrate disease modification. Given the difficulty in modeling symptomatic outcomes in α -syn-based animal models discussed above, uncertainty exists as to what clinical signs and symptoms of a given synucleinopathy may improve following exposure to an α -syn-modulating intervention (e.g., motor, cognitive, autonomic). Modalities like wearable sensors that capture broad and unbiased scopes of function appear particularly well-suited for use in these clinical studies. Demonstration that modulating α -syn produces improvements in a patient's symptomatology or function, not resulting from a drug-drug interaction with co-incidentally administered symptomatic therapies, would constitute PoC.

Choosing the optimal patient population

The known clinical heterogeneity in PD and the variable motor and non-motor manifestations represent a challenge to optimal patient selection. Importantly, the ability to establish initial PoP (or clinical PoC) may depend heavily on the ability to select an experimental population characterized by a nearly uniform underlying biology/pathophysiology or other biological trait—even if such molecularly homogenous population remains *clinically* heterogeneous.

The ideal α -syn patient population carries point mutations, duplication, or triplication of the α -syn gene, *SNCA* [183, 184]. Unlike in sporadic PD, where the pathogenicity of α -syn is assumed, in these populations, the pathogenicity of α -syn is confirmed. Unfortunately, such patients are very rare and are difficult to effectively recruit into clinical trials. Lacking available *SNCA*-PD cohorts, one could argue that a "patient enrichment" strategy using biological

and/or imaging endpoints to identify patients with high expression of pathological α -syn being targeted by a specific therapy, may improve the probability of success in establishing PoP.

One could envision designing a clinical PoP study in PD patients with evidence of synucleinopathy, where the primary goal is to establish that the drug-targeted pathologic α -syn levels are reduced after treatment. Potential trial subjects could be screened for high α -syn levels using analysis of serum, peripheral tissues, and CSF, and potentially via α -syn molecular imaging, once developed. In the absence of a means of identifying the presence of α -syn pathology in living patients, DAT imaging, discussed above, may be used to differentiate Parkinsonian patients with a higher risk of symptom progression [185]. The ability to extend the demonstration of PoP to clinical PoC in a given sub-population, and clinical PoC to the broadest population, will depend on measuring change in clinical status as a result of lowering a relevant α -syn species. As a subset of “responders” may be statistically drowned in an overall negative study, it will be important to anticipate the possibility that a clinical PoC can be confirmed only in a subset of the cohort. Understanding the biological differences between the responders and the rest of the cohort may help fine-tune patient selection for a subsequent phase of development. Implementation of such an approach will require identification of clinical, biomarker and/or genetic indicators associated with responder population for recruitment of patients in the next phase. The identification of such markers to enable personalization of disease-modifying therapy will require that all studies, both interventional and observational, collect harmonized clinical and biomarker measures, and that the field redoubles its efforts to encourage all subjects to provide biospecimens and consent to concurrent and future analyses.

Outcome measures – safety

Non-clinical safety studies are necessary to identify potential liabilities to be monitored in humans and to identify safe starting doses for first-in-human studies. Safety (bio)markers should be sought in non-clinical studies and are particularly useful if they can be used to reduce risk in a clinical program. At present, it is unknown if lowering levels of physiological (“normal”) α -syn species has any inherent liabilities in humans. Optimally, α -syn-based therapeutics should selectively target pathological forms

and spare normal species. Several of the current α -syn-based approaches spare “normal” α -syn species and are specific for α -syn (versus β - and γ -synuclein). Because of our poor understanding of the normal function of the synucleins, no guidance can yet be made regarding “target-mediated” side effects of anti- α -syn therapies.

Outcome measures – proof of principle

The decision to initiate human studies in healthy subjects versus synucleinopathy patients will be specific to each therapeutic modality (small molecule, antibody, gene therapy, antisense oligonucleotide), based on a risk/benefit estimate as determined by non-clinical safety experiments. Therefore, we do not provide a specific “class” recommendation for α -syn-based therapies. It is anticipated that either starting first in human studies in a synucleinopathy patient population or advancing to such a population early in development will be advantageous. The advantages include early assessments of safety issues inherent to the target patient population and enabling assessments of physiologically-based pharmacokinetic/pharmacodynamic relationships that may rely on the presence of underlying α -syn pathophysiology.

Demonstrating a relationship between dose and TE in the brain is important where the TE measure could be specific to a drug candidate. A clear dose-response effect on TE, pharmacodynamic, and safety markers is key to interpretation of results of early stage clinical trials and to the initiation of pivotal trials. Taking advantage of expression of the target α -syn species in the CSF and peripheral biospecimens is an attractive approach to establishing measures for TE. Although extrapolation of exposures and TE from the peripheral to the central compartment has its challenges, evidence for peripheral TE may be highly informative, especially if a relationship is established in animal models and a valid physiologically-based pharmacokinetic/pharmacodynamic model can be built. Additionally, many existing CNS imaging and physiological assessment tools (structural MRI, DTI, fMRI, 2-deoxyglucose imaging, and electroencephalograms) have limited utility as direct TE markers but may be suitable as pharmacodynamic or PoP outcome measures.

Reduction of α -syn protein levels, comparing baseline levels to multiple post-exposure assessments, in biological fluids (serum and CSF), and potentially in accessible cells like peripheral blood mononuclear cells [186, 187] would be an informative outcome. In

order for these modalities to be useful, they must have robust assay metrics (e.g., low test/retest variability). Establishing PoP by demonstrating reductions in α -syn expression in small, relatively short-duration clinical studies will be a critical step, and will help translate PoP into clinical efficacy through the establishment of clinical PoC.

Current challenges in translation proof of principle to proof of concept

In order to support investment in large-scale, long, and expensive pivotal clinical trials, the objective of a PoC trial should be to increase confidence that the previously demonstrated TE/PoP outcomes translate into a clinically meaningful outcome. For PD, such outcomes include assessment of motor function utilizing the Movement Disorders Society Unified Parkinson's Disease Rating Scale (MDS-UPDRS). As such development programs advance, continued assessments of safety and tolerability remain critical.

Ideally, the population upon which PoP was demonstrated can also serve as the population to establish PoC and eventual approval. As discussed above, this may not always be the case, therefore the synucleinopathy patient population is one of the key variables to address in a PoC study. Whereas biological factors may weigh heavily in the choice of patient population to study for PoP, clinical characteristics are critically important in determining an optimal PoC study population. Clinical signs and symptoms differ between synucleinopathies, and inclusion of patients who manifest disease progression with specific clinical characteristics (e.g., predominantly motor versus cognitive versus peripheral/autonomic signs and symptoms) will dictate what clinical outcome measures should be included for use in these studies. For PoC studies, the primary endpoint does not necessarily have to be an approved endpoint, or even the planned pivotal study endpoint (such as MDS-UPDRS, see Table 4). In fact, ideal endpoints for PoC should be more sensitive, more precise, and correlate with or predict outcomes on the primary endpoint planned for pivotal trials (if the primary endpoints are not the same). Planned pivotal study outcome measures should be included in the PoC study, even if the study is underpowered to detect a statistically significant difference between groups. Modeling and simulation techniques should be used to estimate probability of success in future pivotal trials.

Table 4
Alternate outcomes to be considered for alpha-synuclein proof of concept studies

| Modality | Examples |
|--|---|
| Neuroimaging | DAT, VMAT2 or other tracers capable of visualizing structural or functional abnormalities in PD patients |
| Wearable sensors | More granularity and reliable assessments than clinical rating scales (do not need to correlate with or predict change in existent clinical rating scales)* |
| Non-motor endpoints (time to milestones) | Gait, cognition, dysautonomia* |

*If these measures correlate with or predict change on validated clinical rating scales they may be more valuable for PoC studies. DAT, dopamine transporter; PD, Parkinson's disease; PoC, proof of concept; VMAT2, vesicular monoamine transporter 2.

There are, however, several challenges inherent in this approach. If subjects are taking medications for control of disease symptoms, one should carefully consider their effect on chosen outcome measures. One must also consider the current state of validation or available natural history data on novel outcomes (e.g., wearables, new imaging methods) when incorporating them into studies, and must prepare a background dossier to support any go-forward decisions based thereon. There are also several analysis issues. Currently, there is a lack of clarity on the necessary effect size of change in MDS-UPDRS and other outcome scales, as they have not yet been used in a sufficient number of completed trials. We do not yet understand whether existing scales are suitable to be a global measure. Novel composite scales may need to be developed to combine non-motor and cognitive aspects into a single global measure of disease progression in PD. Another outstanding question is whether our statistical methods for declaring PoC are antiquated. Do we really need results of a "delayed start" study to declare PoC, or can modern methods such as modeling and simulation approaches or adaptive and Bayesian study designs maximize our ability to interpret results of studies in relatively small populations, since PoC trials are often underpowered for traditional endpoints? An example of the utility of an adaptive dose randomization strategy with Bayesian statistical approach was reported recently for an AD trial [149]. When coupled with more sensitive clinical outcome measures, if available, it may be possible to reduce the sample size while evaluating PoC at a range of doses.

Many factors influence the choice of clinical outcomes including ease of measurement, metrics of the clinical scales used to assess a particular sign or symptom, and sensitivity to detect change. Progression rate-specific clinical signs and symptoms differ, not only between synucleinopathies but also within given disease state at specific stages of disease (progression can be non-linear). Existing data from longitudinal clinical cohorts for PD such as DATATOP [188], PreCEPT/PostCEPT [189], PPMI [190] and other natural history studies, as well as studies in other synucleinopathies such as MSA and DLB, can be used to model disease progression to optimize trial design.

Although unlikely to be of current utility in PoC study design, the use of prodromal patient populations is likely to become more relevant over time. There are unique challenges in stratification of a *de novo* or prodromal populations. Strategies for how to handle medication use, baseline disease severity, and even accounting for regional practice differences must be devised to minimize interpatient variability. When designing studies to be carried out in prodromal or pre-motor populations, characteristics of the at-risk population will need to be carefully defined. For example, should the initial population be defined by individual symptoms (e.g., RBD) versus symptom complexes (multi-modal approach); obviously genetics and other risk factors will also need to be taken into account and could further help enrich the at-risk population. For the field to be ready to undertake trials in prodromal patients, we will need to improve our understanding of prodromal symptoms and rate of progression to meet existing disease diagnostic criteria in order to reduce the number of patients to be screened for these trials. Conducting a longitudinal, non-interventional clinical study in an enriched prodromal population would generate critical data to support the use of such populations in drug development efforts.

Finally, application of available clinical, imaging, and physiological tools to enrich a given synucleinopathy population will be critical to achieving success in PoC. Stratifying the target synucleinopathy population during randomization or analysis for key clinical variables such as concomitant medication usage, baseline disease severity, genetic factors, and clinical operational variability (e.g., geographic, standard of care) will increase the probability of detecting a clinical effect of the therapeutic intervention under study. In the absence of an α -syn PET ligand, studies are currently relying

on DAT SPECT scans to serve this purpose (see above).

Given the breadth of α -syn-based therapeutic modalities under study and the complexity and variability of the clinical synucleinopathy diseases, PoC trial designs will need to be optimized for each specific therapeutic under study. Sponsors are encouraged to share as much non-competitive α -syn therapeutic PoC trial designs and data as possible, perhaps within clinical trial consortia or harmonized databases such as the Alzheimer's Disease Neuroimaging Initiative (ADNI) [191] and the Critical Path for Parkinson's Initiative [192]. Such collaborative efforts will be critically important to achieve the shared goal of lowering disease burden and improving the lives of existing and future patients suffering from synuclein-based neurodegenerative diseases.

General perspective

That α -syn is the major drug development target for the treatment of human synucleinopathies, and PD in particular, is supported by the depth and mechanistic breadth of the approaches currently being pursued (Table 1). Multiple therapeutics have recently entered clinical trials, and critical human data that will inform all α -syn-based therapeutic development programs is on the horizon. Although these efforts face many profound challenges, including the lack of key tools such as an α -syn-based imaging agent and the inherent difficulty of demonstrating clinical efficacy in slowly progressive neurodegenerative diseases, we remain optimistic that meaningful strides toward the ultimate identification and approval of α -syn-based disease-modifying therapeutics will be made in the near future.

CONFLICT OF INTEREST

KMM is a Senior Advisor to The Michael J. Fox Foundation for Parkinson's Research. She serves on the Scientific Advisory Board and/or provides consulting services to AbbVie, Acumen Pharmaceuticals, Epiodyne, Lysosomal Therapeutics, Neuroinitiative, Pfizer, Sinopia Biosciences and private equity investors. She also serves on the NCATS Advisory Council and CAN Review Board, NIA's Division of Neuroscience Review Committee, and Wellcome Trust Review Board.

JC is an employee and shareholder of Biogen.

PB has received commercial support as a consultant from Renovo Neural, Inc., Cellular Dynamics

International, Axial Biotherapeutics, Roche, Teva Inc, Lundbeck A/S, NeuroDerm, AbbVie, ClearView Healthcare, FCB Health, IOS Press Partners and Capital Technologies, Inc. He is conducting sponsored research on behalf of Roche and Lundbeck A/S. He has ownership interests in Acousort AB.

KDD is an employee of The Michael J. Fox Foundation for Parkinson's Research and has no conflicts of interest to report.

JE is an employee of The Michael J. Fox Foundation for Parkinson's Research and has no conflicts of interest to report.

AJE has received grant support from the NIH, Great Lakes Neurotechnologies and The Michael J. Fox Foundation for Parkinson's Research; personal compensation as a consultant/scientific advisory board member for Abbvie, TEVA, Impax, Acadia, Acorda, Cynapsus/Sunovion, Lundbeck, and USWorldMeds; publishing royalties from Lippincott Williams & Wilkins, Cambridge University Press, and Springer; and honoraria from Abbvie, UCB, USWorldMeds, Lundbeck, Acadia, the American Academy of Neurology, and the Movement Disorders Society.

SJH is an employee The Michael J. Fox Foundation for Parkinson's Research and has no conflicts of interest to report.

MJ has no conflicts of interest to report.

JL is a full time employee of Eisai Inc, USA. He owns shares in AstraZeneca and Merck Co.

WM receives or received funding from the European Union, The Michael J. Fox Foundation for Parkinson's Research, Robert Bosch Foundation, Neuroalliance, Lundbeck and Janssen, and holds part of a patent for the assessment of dyskinesias (German patent office, 102015220741.2). He received speaker honoraria from GlaxoSmithKline, Abbvie, UCB, Licher MT and Rölke Pharma, and was invited to Advisory Boards of Market Access & Pricing Strategy GmbH and Abbvie.

LM is an employee The Michael J. Fox Foundation for Parkinson's Research and has no conflicts of interest to report.

ANR is an employee of The Michael J. Fox Foundation for Parkinson's Research and has no conflicts of interest to report.

AJS is an employee of the University of British Columbia and is supported by a Canada Research Chair and by research grants from the Michael J. Fox Foundation and Weston Brain Institute. He is Deputy Editor of Movement Disorders. He has received consulting and speaking honoraria from AbbVie and

is an advisor for Cellular Dynamics International and on a Data Safety Monitoring Board for Voyager Inc.

DMW is an Advisor to The Michael J. Fox Foundation for Parkinson's Research.

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