Animal models of α-synucleinopathy for Parkinson disease drug development

James B. Koprich[1,2], Lorraine V. Kalia[1,3–5] and Jonathan M. Brotchie[1,2]

Abstract | A major challenge in Parkinson disease (PD) will be to turn an emerging and expanding pipeline of novel disease-modifying candidate compounds into therapeutics. Novel targets need *in vivo* validation, and candidate therapeutics require appropriate preclinical platforms on which to define potential efficacy and target engagement before advancement to clinical development. We propose that α-synuclein (α-syn)-based mammalian models will be crucial for this process. Here, we review α-syn transgenic mouse models, viral vector models of α-syn overexpression and models of 'prion-like' spread of α-syn, and describe how each of these model types may contribute to PD drug discovery. We conclude by presenting our opinion on how to use a combination of these models through the late-stage preclinical, proof-of-principle investigation of novel therapeutics.

[1]*Krembil Research Institute, Toronto Western Hospital, University Health Network, 60 Leonard Ave, Toronto, Ontario M5T 2S8, Canada.*
[2]*Atuka, Inc., 100 King St W, Suite 5600, Toronto, Ontario M5X 1C9, Canada.*
[3]*Morton and Gloria Shulman Movement Disorders Clinic and the Edmond J. Safra Program in Parkinson's Disease, Division of Neurology, Department of Medicine, Toronto Western Hospital, University Health Network, 399 Bathurst St, Toronto, Ontario M5T 2S8, Canada.*
[4]*Tanz Centre for Research in Neurodegenerative Diseases, University of Toronto, 60 Leonard Ave, Toronto, Ontario M5T 2S8, Canada.*
[5]*Division of Neurology, Department of Medicine, University of Toronto, 200 Elizabeth St, Toronto, Ontario M5G 2C4, Canada.*

Correspondence to J.B.K.
j.koprich@atuka.com

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Parkinson disease (PD) is the most common neurodegenerative movement disorder, with an estimated worldwide prevalence of 570 per 100,000 among people aged 50 and older and that increases with age[1]. The classical motor features of parkinsonism (bradykinesia, muscular rigidity, rest tremor, and postural and gait impairment) are prominent symptoms of PD, but non-motor features (including cognitive impairment, psychiatric symptoms, sleep disorders, autonomic dysfunction, pain and fatigue) also frequently occur and affect quality of life[2]. Single gene mutations are a rare cause of PD; the aetiology of sporadic PD remains unknown but probably involves the complex interplay of genetic and environmental factors. Irrespective of aetiology, all cases of PD show loss of dopaminergic neurons in the substantia nigra pars compacta (SN), and most show Lewy pathology consisting of Lewy bodies and Lewy neurites using current histological methods. Importantly, α-synuclein (α-syn) is the major component of the Lewy body[3]. Thus, it is possible that diverse initial processes converge downstream on α-syn as a key player in disease pathogenesis.

Animal models of the molecular pathology of PD will provide valuable insight into potential new targets for disease intervention. More traditional, toxin-based models of PD based on killing dopaminergic neurons (that is, the 6-hydroxydopamine (6-OHDA) rat, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse and the MPTP primate) have proved useful in developing treatments for PD symptoms and in investigating side effects associated with dopamine-replacement therapies. However, they have not succeeded in identifying agents that can slow, halt or reverse disease course. Only 2 of the more than 50 candidates clinically tested after assessment in toxin-based models are still considered to have clinical disease-modifying potential (namely, GM1 ganglioside[4,5] and rasagiline[6,7], which were tested using MPTP models). The PD toxin models are acute, rapid and do not model the molecular pathology of PD. They kill dopamine neurons by mechanisms that may not be reflective of PD and produce a dopamine-loss phenotype without any progressing evolution of pathology. Although these models have proved extremely useful for defining potential symptomatic benefits, these failings probably underlie their poor track record in predicting disease modification.

Modelling the core, progressive, degenerative process — α-syn-driven dysfunction and death[8] — may provide a more reliable means of prioritizing compounds for clinical development. Indeed, α-syn-based aggregations represent a common pathology that links many genetic forms of PD (BOX 1), making α-syn an attractive protein to represent a final common pathway or point of convergence of multiple pathologies, and thus an appealing basis for animal model development and drug candidate screening. The focus on α-syn should not detract attention from research into other PD-related loci, which may identify upstream targets of toxic α-syn species production and thereby mitigate or prevent against further α-synucleinopathy. Furthermore, therapies aiming to

Box 1 | **The genetic link between α-synuclein and Parkinson disease**

Whether contributing to risk or through direct Mendelian inheritance, the list of genes and loci implicated in Parkinson disease (PD) continues to grow. Notable advances in understanding PD pathogenesis have emerged from such genetic discoveries. There are as many as 23 confirmed monogenic conditions in which parkinsonism is a consistent and predominant feature[172]. Of these, mutations in at least five genes, *SNCA*[173,174], *LRRK2* (REFS 175,176), *PINK1* (REFS 177,178), *PRKN*[179–181] and *PARK7* (REFS 182,183), can be considered directly related to PD pathogenesis (see the table). The post-mortem brains of most, but not all, individuals with PD who carry these mutations (subsets of *PRKN*-related and *LRRK2*-related cases being the exceptions[184,185]) contain Lewy pathology, including a recently described *PARK7*-related case[186], suggesting that these distinct pathogenic causes of PD all converge on α-synuclein (α-syn) aggregation.

The first missense mutation in the *SNCA* gene that was associated with autosomal-dominant PD was A53T[187]. Since then, further *SNCA* missense mutations (A30P[188], E46K[189], H50Q[190,191], G51D[192] and A53E[193,194]), as well as multiplications of the *SNCA* locus, have been identified as genetic causes of PD[195,196]. Moreover, genome-wide association studies have shown a link between *SNCA* and sporadic PD[197–201]. *SNCA* encodes the protein α-syn, which is the major component of Lewy pathology; no other gene has been so intimately linked to PD.

Gene	Protein	Pathogenic mutations
Autosomal-dominant variants		
SNCA	α-Syn	• Missense mutations: A30P, E46K, H50Q, G51D, A53E and A53T • Multiplications: duplications and triplications
LRRK2	Leucine-rich repeat serine/threonine-protein kinase 2	• Missense mutations: N1437H, R1441C, R1441G, R1441H, Y1699C, G2019S and I2020T
Autosomal-recessive variants		
PRKN	Parkin	• Most common: exon rearrangements (exon deletions or multiplications) • Other: missense mutations, nonsense mutations, small deletions or insertions and splice-site alterations
PINK1	PTEN-induced putative kinase protein 1	• Most common: missense mutations and nonsense mutations • Other: exon rearrangements (exon deletions or duplications)
PARK7	DJ1	• Most common: missense mutations and exon rearrangements (exon deletions) • Other: splice-site alterations

Bradykinesia
Slowness of movement and decrement in amplitude or speed (or progressive hesitations or halts) as movements are continued.

1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine
(MPTP). A neurotoxin that, when injected into most animals, will produce a selective lesion of the dopamine system that can be used to model nigral degeneration.

Ganglioside
Sialic acid-containing glycosphingolipid differentiated by the structure of its carbohydrate chains. Gangliosides are primarily localized in plasma membranes and have prominent roles in various cell functions.

reduce levels of harmful α-syn could be useful in other α-synucleinopathies (such as multiple system atrophy or dementia with Lewy bodies) (BOX 2).

This Review discusses vertebrate animal models of PD based on α-syn (as only vertebrates contain endogenous α-syn), with a specific focus on their application to therapeutic development. We critique rodent transgenic models that overexpress α-syn, viral vector-based overexpression of α-syn in rodents and primates, and models of α-syn spread based on inoculation with synthetic or recombinant-based preformed fibrils (PFFs). Cellular and non-mammalian models (such as those in *Caenorhabditis elegans*, *Drosophila melanogaster*, yeast or zebrafish) are not discussed, nor will we provide an in-depth review of physiological and pathophysiological roles of α-syn (for reviews on these topics, see REFS 9–11).

Physiology and pathophysiology

To understand the role of α-syn in PD pathogenesis, and thereby develop appropriate models, we must first understand the physiological role of monomeric and possibly tetrameric or multi-oligomeric α-syn[12] and how loss of this function might affect otherwise healthy neurons. We must also understand how abnormal α-syn, whether mutated or higher-order species (that is, oligomers or aggregates), might gain function to initiate degenerative processes. Both gain-of-function and loss-of-function mechanisms probably contribute to disease presentation and are potential therapeutic targets.

Physiological functions of α-synuclein. The normal functions of α-syn have yet to be completely elucidated; however, it seems to have a role in exocytosis[13] and synaptic vesicle-mediated release of neurotransmitter (FIG. 1). Indeed, mice lacking the gene encoding α-syn (*Snca*) show impaired stimulated release of dopamine[14], mice lacking both α-syn and β-syn show reduced brain levels of dopamine (by 20%)[15], and mice lacking all three synuclein homologues (α, β and γ) show reduced excitatory synapse size and reduced synaptic function with age[16].

More specifically, monomeric α-syn interacts with SNARE (soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor) complexes, which are directly involved in vesicle fusion, exocytosis and, in the nigrostriatal pathway, dopamine release[17,18].

Protofibrils, fibrils and oligomers
Different α-synuclein conformers associated with the pathogenesis of Lewy body diseases, including Parkinson disease. Fibrils in particular are found in abundance in Lewy bodies.

Rapid eye movement (REM) sleep behaviour disorder
(RBD). A parasomnia characterized by abnormal or disruptive behaviours (such as shouting, gesturing or kicking) that occur during REM sleep and are often related to dream enactment.

Overexpression of α-syn has been shown to reduce dopamine release in transgenic mice[19,20] and viral vector models[21]. In bacterial artificial chromosome (BAC) transgenic mice overexpressing α-syn, synaptic vesicles are abnormally clustered and intervesicle distances are reduced[22,23], possibly reflecting a role of α-syn in vesicular recycling and explaining how pathological α-syn may affect transmitter release. Physiologic α-syn is also involved in intracellular trafficking within the endoplasmic reticulum–Golgi network, at least in *D. melanogaster* and yeast, where α-syn overexpression disrupts cargo trafficking[24,25]. Such results have supported the use of yeast in drug discovery programmes aimed at finding pathways affected by α-syn overexpression, engageable by re-purposing existing drugs and testable in mammalian models[26–28]. Restoring normal α-syn function might rescue neurotransmitter release and intracellular trafficking in neurons not destined to die early in the disease process, and could thereby improve function. Such a therapeutic could have a large impact, as these neuronal populations are the most likely to be affected by early intervention.

Functions of pathological α-synuclein. When α-syn shifts from its monomer form to higher-order structures (protofibrils, fibrils and oligomers), its function also shifts from engaging in normal cell physiology towards involvement in toxic processes (FIG. 1). Protofibril and fibril structures seem to be the most toxic[29], and their production, truncation and maintenance are probably fundamental to PD pathology. In PD and other α-synucleinopathies, disease severity correlates with the propensity of α-syn to misfold and form these higher-ordered structures[11].

Whereas some mutations and multiplications of *SNCA* render the encoded α-syn more likely to form pathological structures, sporadic cases of α-syn transformations are less well understood. Similarly, it is not yet known how single-nucleotide polymorphisms (SNPs) in *SNCA* identified in genome-wide association studies (GWAS) contribute to increased risk of sporadic PD. The most studied region of the brain in PD, the nigrostriatal dopaminergic system, may offer some clues. A9 dopaminergic neurons are particularly vulnerable to cell death in PD and in experimental models of α-synucleinopathy[30]. These neurons endure an exceptionally high electrophysiological workload[31], support heavily arborized axons[32] and have very thinly myelinated projections[33], and this burden is exacerbated with ageing. Moreover, they experience environmental insults, age-related impairment of autophagy and changes in the α-syn protein that potentially lead to α-syn accumulation and formation of toxic higher-order α-syn species that further increase vulnerability[34–36]. Misfolded α-syn impairs protein clearance and degradation[37,38]; promotes endoplasmic reticulum stress[24–26], mitochondrial dysfunction and oxidative stress[39,40]; overwhelms calcium-buffering capacity[41]; impairs axonal transport[42]; and increases neuroinflammation[43–45] (FIG. 1).

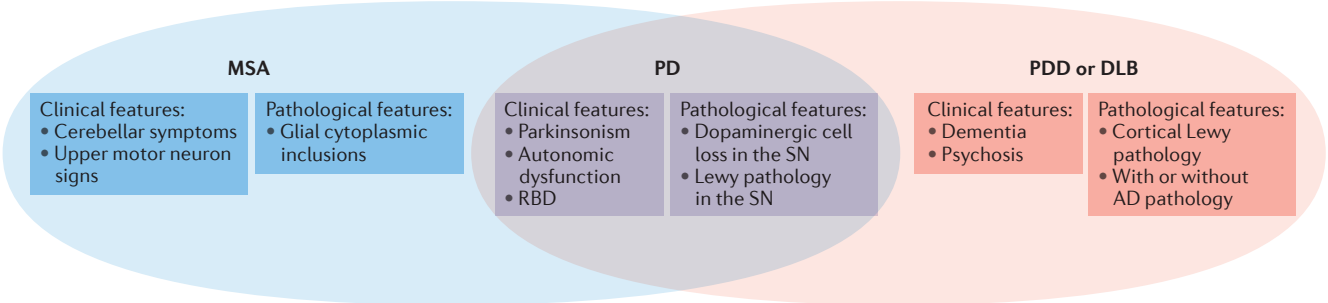
Transgenic models

Many transgenic mice expressing α-syn have been generated to try to model PD in order to study α-syn pathobiology and evaluate potential therapeutics (TABLE 1). Efforts have been made to commercialize and make publicly available some of the most popular lines in the hope of standardizing application of the models and facilitating

Box 2 | **The α-synucleinopathies**

Several neurodegenerative diseases — namely, Parkinson disease (PD) and PD dementia (PDD), dementia with Lewy bodies (DLB) and multiple system atrophy (MSA) — are associated with abnormal accumulation of α-synuclein (α-syn) in the brain and hence are collectively termed 'α-synucleinopathies'. These diseases have distinct, as well as overlapping, features that seem to fall along a spectrum (see the figure).

PD, the most common α-synucleinopathy, is clinically characterized by parkinsonism (that is, bradykinesia, muscular rigidity, rest tremor, and postural and gait impairment), as well as non-motor features, including cognitive impairment, autonomic dysfunction and rapid eye movement (REM) sleep behaviour disorder (RBD). PD is defined by the loss of dopaminergic neurons and the presence of Lewy pathology in the substantia nigra pars compacta (SN). PDD is diagnosed when dementia occurs after the onset of parkinsonism. DLB is the second most common neurodegenerative dementia after Alzheimer disease (AD). Its core clinical features are parkinsonism, cognitive fluctuations and visual hallucinations. Dementia occurs before or concomitantly with parkinsonism in DLB (a major distinguishing feature from PDD). The pathology of PDD and DLB can be pure Lewy pathology with cortical Lewy bodies, or a mixed pathology with Lewy bodies, as well as amyloid-β plaques and neurofibrillary tangles (neuropathologic features of AD). MSA is clinically characterized by autonomic dysfunction with parkinsonism or cerebellar symptoms. Lewy bodies can be seen in MSA, but the presence of α-syn aggregates in oligodendrocytes, called glial cytoplasmic inclusions, is required for a definitive pathological diagnosis. Other neurodegenerative diseases associated with Lewy pathology include Gaucher disease and additional lysosomal storage disorders, a subset of neurodegeneration with brain iron accumulation (NBIA) disorders and AD.



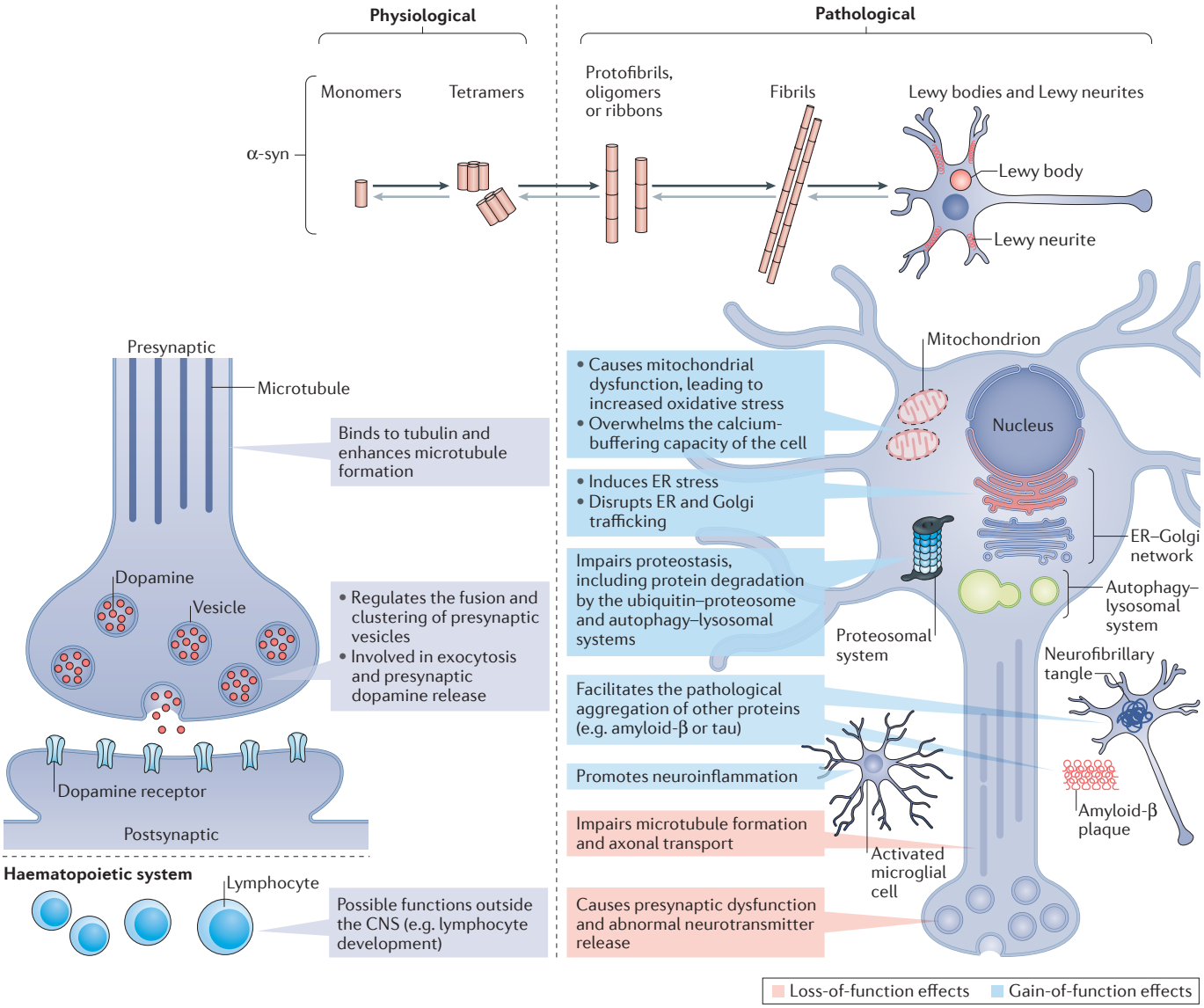


Figure 1 | **Proposed physiological and pathological roles of α-synuclein.** α-Synuclein (α-syn) exists in various forms, and its diverse functions may depend on its form. Monomeric and possibly tetrameric α-syn are probably the native forms of the α-syn protein that function in the physiological or non-disease state. Physiological functions of α-syn include roles in regulating exocytosis — such as in vesicle–membrane fusion, vesicle clustering and presynaptic neurotransmitter release in dopaminergic neurons — and in enhancing microtubule formation through interactions with tubulin. α-Syn is also expressed outside of the CNS, where its functions still remain to be elucidated but may include a role in lymphocyte development in the haematopoietic system. Protofibrils, oligomers, ribbons and fibrils are hypothesized to be the pathological or toxic forms of α-syn in Parkinson disease (PD) and other α-synucleinopathies. Lewy bodies and Lewy neurites may themselves be toxic or may serve as a reservoir of misfolded α-syn. The toxic forms of α-syn can result in loss-of-function effects, such as impaired neurotransmitter release and disrupted microtubule formation. Gain-of-function effects due to toxic α-syn include mitochondrial dysfunction, endoplasmic reticulum (ER) stress, impaired proteostasis, enhanced neuroinflammation and pathological aggregation of other proteins.

effective comparisons of results across laboratories. Most of the transgenic models overexpress human wild-type α-syn (modelling *SNCA* multiplications), or human A53T, A30P or mutant α-syn (modelling *SNCA* missense mutations). A few express α-syn with modifications (such as C-terminal truncation[46–48]) expected to increase toxicity, and at least two models overexpress mouse α-syn[49,50].

The most commonly used models drive pan-neuronal transgene expression under the control of the human platelet-derived growth factor subunit B (*PDGFB*) promoter, mouse thymus cell antigen 1 (*Thy1*) promoter or mouse *Prnp* promoter. The rat tyrosine hydroxylase (*Th*) promoter has been used to limit α-syn expression to catecholaminergic neurons, including dopaminergic neurons in the SN[48,51–53]. Using tetracycline-controlled transcriptional activation, α-syn transgene expression in forebrain neurons (under control of the calcium/calmodulin-dependent protein kinase type II subunit-α (*CamkIIa*) promoter)[54,55], midbrain dopaminergic neurons (pituitary homeobox 3

Tetracycline-controlled transcriptional activation
Inducible gene expression in which transcription of a target transgene is reversibly turned on or off in the presence of tetracycline or a derivative (such as doxycycline).

Table 1 | **Features of transgenic mouse models and viral vector-based α-synuclein models**

Promoter or virus	α-Syn	Age or time from AAV injection (months)							Refs
		Brain α-syn aggregation	Peripheral α-syn	SN cell loss	Striatal abnormalities	Brain gliosis	Motor features	Non-motor features	
Mouse models									
Thy1	hWT	4.5*	5	—	6	6	2	3	66,109,205–207
	hA53T	6	NR	NR	3	NR	4.5	NR	50,98
	hA30P	8	NR	NR	—	8	8	12	71,97,208
PDGFB	hWT	3*	NR	—	12	12	9	12	109,112,209,210
Prnp	hA53T	≥3	≥3	—	NR	NR	2	6	67,82,101
	hA53T	≥4	NR	—	—	≥10	≥10	NR	73
	hA30P	—	NR	—	—	4	6	NR	65,96
	hE46K	≥16	NR	NR	NR	≥16	≥16	NR	72
SNCA	hWT	—	6	—	—	NR	6	3	59
	hA53T	—	NR	18	3	—	18	3	60
Th	hA53T+A30P	—	NR	22	2	NR	7	NR	51,92
	$hA53T_{1-130}$	—	NR	2	2	—	2	NR	47
	hWT_{1-120}	12*	3	—	3	12	18	NR	46
Rat models									
AAV1/2, AAV2/6 or AAV2/7	hWT or hA53T	1*	¶	1	1	1	1	NR	126,127,136
Primate models									
AAV1/2	hA53T	4*	¶	4	4	NR	NR	NR	154
AAV2 or AAV2/5	hWT or hA53T	12*	¶	12	12	12	12	NR	151,152

α-Syn, α-synuclein; AAV, adeno-associated virus; hA30P, human A30P mutant; hA53T, human A53T mutant; hE46K, human E46K mutant; hWT, human wild type; NR, not reported; *PDGFB*, gene encoding platelet-derived growth factor subunit B; *Prnp*, gene encoding prion protein; SN, substantia nigra; *SNCA*, gene encoding α-syn; *Th*, gene encoding tyrosine hydroxylase; *Thy1*, gene encoding thymus cell antigen 1; —, absent. *α-Syn inclusions are found in (but may not be limited to) the SN. ¶α-Syn expression is targeted to the SN.

(*Pitx3*) promoter)[56] or all neurons (*Prnp* promoter)[57,58] can be switched on after postnatal development (to preclude early compensation for α-syn overexpression) or off after symptom onset (to assess phenotype–expression correlations). BAC models with upstream promoter elements have also been developed, often on a mouse *Snca*$^{-/-}$ background, to drive expression of human *SNCA* at endogenous levels and to avoid potential confounding interactions with endogenous mouse α-syn[20,59,60]. A transgenic model in which the mouse *Snca* promoter drives human α-syn expression has also been generated[61–63].

Features of transgenic mouse models. α-Syn forms intracellular inclusions in many but, perhaps surprisingly, not all α-syn-overexpressing transgenic mice[47,48,51,53,64,65]. Models using *Th* or *Snca* promoters tend to lack α-syn inclusions, but no other factors (such as mutations or expression levels) are clearly related to aggregation propensity. The anatomical distribution of α-syn inclusions also varies widely among models, even among those with the same promoter. α-Syn inclusions, in the models that exhibit them, have biochemical characteristics similar to those of aggregated α-syn in human PD (for example, they contain pSer129 and are ubiquitylated, thioflavin S positive, proteinase K resistant and detergent insoluble[66–71]). However, only the model expressing human E46K mutant α-syn displays inclusions with the 'core and halo' morphology of classical Lewy bodies in human PD[72]. Neuroinflammation is often observed in areas with substantial α-syn aggregation[67,71–74]. Importantly, some models have minimal transgene expression in the SN and thus, in contrast to the human disease, no α-syn inclusions. Whereas in human PD α-syn inclusions in the spinal cord are observed but are not thought to mediate motor symptoms of the disease[75,76], in some transgenic mice models such inclusions probably underlie their motor phenotypes (see below).

The Braak hypothesis of the spread of pathological α-syn[77,78] (see below) proposes that α-syn aggregates form in the periphery (for example, in the olfactory bulb or enteric nervous system) in early stages of PD, before α-syn aggregation in the brain. As a result of this hypothesis, some transgenic mice have been examined to assess whether they could be useful models to test early administration of disease-modifying therapies.

pSer129
Phosphorylation site associated with toxic forms of α-synuclein.

'Core and halo' morphology
The classical morphology of a nigral Lewy body: a spherical cytoplasmic inclusion with a hyaline eosinophilic core and a narrow, pale-stained halo.

Indeed, in some models, α-syn inclusions are observed in olfactory bulb[46,66,79] or gut[68,80–82], as well as retina[83], sciatic nerve[74], skin[68], adrenal gland[79] and heart wall[68]. No data from transgenic mice provide support for spontaneous 'spreading' of pathological α-syn; one study found that peripheral and brain α-syn pathology temporally coincide[82], and most other studies only examined peripheral α-syn at time points after α-syn inclusions appear in the brain. Moreover, whether peripheral α-syn will be a useful biomarker of early disease process in these preclinical models is not known. There are conflicting reports on specificity of peripheral α-syn for PD in humans; for example, some studies have reported similar α-syn accumulation in the gut of patients with PD and of healthy controls[84–86].

Unexpected for a protein associated with considerable toxicity *in vitro*, transgenic overexpression of α-syn in mice does not prominently or consistently lead to neurodegeneration (TABLE 1). Characterization of all the early transgenic mouse models revealed that there was no dopamine neuron loss in the SN. Neurodegeneration in PD is an age-related process, and the lifespan of mice may be too short for α-syn-mediated neurodegeneration to occur. A few models showed neuronal loss in extranigral regions, including the neocortex and hippocampus[54,87–89], which are PD relevant, but also in the spinal cord, which is less characteristic of PD[50,74,90]. Some models initially reported to have no SN neurodegeneration, and some newer transgenic models, were later found to show decreases in SN cell number (on average, 35% lower than the SN cell number in controls) at 8 months or later[56,60,91,92]. Some models without SN cell loss exhibit abnormalities in the striatum, such as reduced TH staining, decreased dopamine levels or release, and increased dopamine transporter (DAT) expression[43,46,48,51,93–95], suggesting early nigrostriatal dysfunction. These markers, as opposed to dopamine neuron loss, may therefore be more robust outcome measures in preclinical studies.

Bradykinesia, rigidity, tremor, and postural and gait impairment define the motor parkinsonian syndrome. Motor impairment is also commonly observed in α-syn transgenic mice (TABLE 1), even in those without α-syn inclusions or nigrostriatal dysfunction[96]; for example, α-syn transgenic mice may show decreased spontaneous locomotion and rearing in the open-field test, reduced time on the rotarod and gait with reduced stride length. Although behavioural assays for parkinsonism in mice are not well established, these findings could respectively represent bradykinesia, postural instability and shuffling gait characteristic of PD — although, if these are not reversed by anti-parkinsonian agents, it would be difficult to accept them as such. A few models unexpectedly demonstrate hyperactivity[79,97,98], which may reflect anxiety-like behaviour (see below). Motor impairment is often found to progress with age, as expected for a neurodegenerative process. Turning off α-syn expression in conditional models reduces the rate of decline in rotarod performance, demonstrating correlation of motor dysfunction with α-syn expression[57].

Unfortunately, motor phenotypes in an individual model are often variable, limiting the utility of those phenotypes as preclinical outcome measures. Severity of the motor phenotype also differs across models. Mice with *Prnp* promoter-driven α-syn expression generally have the most-severe motor abnormalities. In these animals, which often have considerable spinal cord pathology, weakness due to motor neuron dysfunction (as opposed to parkinsonism) seems to be the most prominent feature, with animals eventually becoming paralysed. Importantly, it is uncertain whether the less-severe motor impairments in other transgenic animals are due to nigrostriatal dysfunction, as responsiveness to dopaminergic treatment is infrequently evaluated[47,99], and thus they may not truly model parkinsonian phenotypes.

Extranigral expression of α-syn in transgenic animals provides the potential for modelling non-motor features of PD. This could have important implications for development of symptomatic therapies for non-motor symptoms in PD and for preclinical testing of putative disease-modifying therapies, which are expected to be most effective if administered in the prodromal phase (when only non-motor symptoms are present[100]). Cognitive impairment in rodents, which is typically evaluated using the Morris water maze or fear conditioning, is observed in models with human A53T α-syn under the *Prnp* promoter[101], human wild-type or A30P α-syn expression under the *Thy1* promoter[97,102] or tetracycline-inducible human wild-type or A53T α-syn expression under the *CamkIIa* promoter[55,57]. Anxiety-like behaviours, which are mostly assessed with the open-field test and elevated plus maze, may be increased[79,98] or decreased[101,103–105] in such models, even in the same transgenic animal[79,101,103,104], and so are inconsistent features. Sleep disorders have not yet been sufficiently studied in α-syn transgenic mice, but one report describes circadian dysfunction[106]. Olfactory impairment[66,95,107] and constipation (reduced stool frequency, lower stool water content)[59,68,80,81,108] occur, although not before motor dysfunction, indicating that they are not prodromal features.

Use of transgenic mice in preclinical development. α-Syn transgenic mouse models have strong construct validity, as they are based on human genetic data implicating mutations in or overexpression of *SNCA* in PD. These models collectively exhibit key features of PD, including α-syn inclusions, nigrostriatal dysfunction, motor phenotypes and non-motor features, and so possess face validity but with several caveats (see above and TABLE 1). The mouse model that seems to have the best face validity expresses human wild-type α-syn under the control of the *Thy1* promoter[109]; this animal exhibits early α-syn aggregation with inclusions in disease-relevant brain regions including the SN, olfactory bulb and cerebral cortex but not spinal cord; loss of TH-positive fibres in the striatum; early motor dysfunction; cortical neuronal loss and cognitive impairment with age; and non-motor features (namely, olfactory impairment and constipation). Whereas the α-syn pathology in this model takes approximately 4 months to develop, many of the other end points

Morris water maze
A commonly used behavioural test for mouse or rat that assesses spatial learning and memory.

Construct validity
The ability of a model to measure what it is intended to measure.

Face validity
The ability of a model to reproduce the clinical and pathological features of the human disease.

require up to 6 months, which might be considered too long for evaluating potential therapeutics. The ability of transgenic models to predict whether drugs will have therapeutic value in humans (that is, their predictive validity) remains to be determined. As described above, very few models seem to recapitulate the symptomatic benefit of currently standard dopaminergic therapies. Several putative disease-modifying therapies, including small molecules[87,94,110,111], immunotherapy[102,112–115] and virus-based gene therapy[116,117], have recently been tested in transgenic mouse models, with the presumption or hope that they will have better predictive validity than toxin models. Some of the putative pharmacotherapies that demonstrated efficacy in transgenic models are currently being evaluated in clinical trials — including *N*-acetylcysteine[94], nilotinib[110] and HMG-CoA (3-hydroxy-3-methyl-glutaryl-coenzyme A) reductase inhibitors[87]. Passive immunotherapies and active immunotherapies that target α-syn also underwent preclinical evaluation in transgenic mouse models and are now in clinical trials[102,112,115], which will help to determine the predictive validity of α-syn transgenic models.

The most appropriate use of transgenic mouse models in preclinical development seems to be to assess whether a putative therapy reduces or clears α-syn inclusions in neurons. Nigrostriatal deficits such as post-mortem levels of dopamine, for example, could be used as secondary outcomes. The utility of motor phenotypes as outcome measures seems to be limited because of their variability and questionable relevance to dopamine dysfunction. Recent preclinical studies have relied on more than one rodent model, often testing drug effects on α-syn aggregation using a transgenic mouse model and on SN neurodegeneration or motor abnormalities using a viral vector-based rat model[87,94,110]. Models with cortical neuron loss and cognitive impairment could potentially be used for preclinical testing of drugs intended for PD dementia and/or dementia with Lewy bodies. Further investigation of the non-dopaminergic features of these models is needed to determine whether they will be useful in testing potential therapies for non-motor symptoms. Similarly, the neuroinflammatory features of these models must be better characterized before using them to test anti-inflammatory treatment strategies. More research into peripheral α-syn in humans and transgenic mice is needed, but these mouse models have the potential to help in biomarker discovery.

Transgenic non-human primate models. A recently reported transgenic model of PD in macaques was generated using lentiviral transfer of (human) *SNCA* into oocytes[118]. Niu *et al.* showed (albeit in only 3 animals) that behavioural abnormalities can be expressed as early as 1.5 years of age compared with non-transgenic age-matched controls. Such deficits include reduced finger dexterity and coordination in a motor task, and the oldest animal (2.5 years old) showed signs of cognitive dysfunction in a recognition test. Despite the small sample size and lack of parallel controls, these results support the further development of transgenesis to model the α-synucleinopathy of PD in non-human primates (NHPs).

Viral vector-based models

Viral vector-mediated overexpression of α-syn was the first approach to produce a robust α-syn-driven degeneration of dopaminergic neurons in mammals[119,120]. This approach was catalysed by the discovery of α-syn as a potential causative factor in PD pathology and has stood the test of time and evolved dramatically — being used to deliver α-syn in rats, mice, pigs and NHPs. As α-syn is the main component of the Lewy body, and missense mutations or multiplications of *SNCA* produce PD, these models clearly have good construct validity. The face validity is also strong, with dopaminergic degeneration, parkinsonian behavioural deficits and/or a Lewy-like α-synucleinopathy produced to varying degrees (TABLE 1).

Considerations of different viruses. Initial reports in 2002 showed that lentiviral[121] and adeno-associated virus 2 (AAV2)[120] delivery of the gene encoding α-syn to the rat SN could directly cause neurodegeneration of the nigrostriatal system. Shortly after, models in which lentiviral co-delivery of both *PRKN* (which encodes parkin) and human mutant *SNCA* were used to show neuroprotective effects of the former[122]. AAVs have since become the 'gold standard' for such approaches, as they have distinct advantages over lentiviruses. Despite these advantages, variability in the amount of degeneration (21–80% nigral neuron loss) and the latency to degeneration (8–52 weeks)[120,123–125] remain considerable issues. Newer generations of hybrid serotype adeno-associated viruses based on AAV2 produce greater degeneration with less variability (40–80% nigral neuron loss) and in shorter time frames (3–26 weeks), and produce behavioural deficits that are reversible by L-DOPA (3,4-dihydroxyphenylalanine)[126,127] (which, as described above, is a prerequisite for accepting that motor behaviours are directly relevant to parkinsonism).

Towards an optimal preclinical virus-based delivery model. Although these latest-generation models are impressive in terms of the amount and rapidity of nigral cell loss, the relevance to PD pathophysiology must be considered. This is an especially important point when considering modelling early stages of PD. At the time of 1 year post diagnosis, patients with PD may retain up to 90% of their SN dopamine neurons and 50% of their striatal dopaminergic innervation[128], although earlier reports put age-adjusted estimates closer to 70% SN neurons[129–131] (for a review, see REF. 132); thus, despite presenting with clinical symptoms, a substantial amount of cellular substrate remains available for therapeutic intervention. The phenotype in patients with early PD may therefore be more driven by an active functional α-syn pathology that interferes with normal cell physiology rather than by overt degeneration. The toxin models, such as the 6-OHDA rat, fail to model any interference with α-syn physiology in otherwise viable neurons; in these models, spontaneous behavioural deficits required >80% loss of striatal dopaminergic innervation and >50% loss of SN dopamine neurons[133,134]. Not only does this not model the situation in early PD, but such models leave little anatomical

Passive immunotherapies
Exogenous antibodies specific to an antigen (such as α-synuclein) that are delivered by intravenous, subcutaneous or intraperitoneal injection.

Active immunotherapies
Vaccinations that activate the immune system of the body to produce endogenous antibodies specific to an antigen (such as α-synuclein).

Hybrid serotype adeno-associated viruses
Adeno-associated viruses produced to express two viral serotypes on their particle surface.

substrate in which to show disease-modifying drug effects (although they are ideally suited for studying dopamine replacement or symptomatic therapies). To model this situation more accurately, newer generations of AAV α-syn-based models therefore need to better recapitulate this stage of the disease rather than result in frank degeneration (which limits the neuronal substrate in which to evaluate, for example, α-syn-clearing strategies).

In our opinion, a target model for early PD is one in which: >95% of SN neurons are transfected; α-syn pathology manifests as Lewy-like inclusions; neurites develop a dystrophic morphology; the α-syn transgene is transported to striatal terminals; a behavioural phenotype develops; there is a 25–40% loss of SN dopaminergic neurons; and there is a 30–50% loss of markers of striatal dopamine function (such as dopamine, TH or DAT). This can be achieved by delivery of AAV1/2 (A53T α-syn, under a cytomegalovirus (CMV) or chicken β-actin (CBA) promoter)[135,136], AAV2/6 (wild-type α-syn, under a synapsin 1 promoter)[126] or AAV2/7 (A53T α-syn, under a CMV or synapsin 1 promoter)[127].

Most of the AAV-based models produce α-syn aggregations, which, when evaluated, contain pSer129 (REFS 126,137–139) and ubiquitin[127,140,141]. Projections to the striatum show dystrophy[120,135,136,142] and axonal transport deficits[142,143]. Useful primary measures to evaluate the efficacy of therapeutic candidates include: striatal dopamine levels; nigral dopaminergic neuron numbers (total, and with and without α-syn); DAT levels; behavioural asymmetry; and striatal α-syn levels. Deficits in these end points have been repeatedly shown in AAV-based models and have been ameliorated by various treatments, including autophagy enhancement[144]; inhibitors of Janus kinase (JAK)–signal transducer and activator of transcription (STAT) signalling[145]; glucocerebrosidase[117]; the calcineurin inhibitor FK506 (REF. 146); and α-syn-targeted antibodies[147]. These end points, combined with analyses of the biochemistry of the α-syn aggregates, suggest that these models offer good face validity.

Viral vector-based delivery outside the nigrostriatal system. To date, α-synucleinopathy after viral vector delivery of α-syn genes has not been found to spread beyond the target pathway or region (for example, the SN, where pathology is presumed to drive most PD motor symptoms). Although this enables direct examination of an anatomical projection, it does not model the widespread, multisystem, evolving pathology representative of PD pathogenesis. Non-nigral pathology probably underlies much of the non-motor components of PD. The effects of α-synucleinopathy in regions outside the SN are not necessarily the same as those in nigral dopaminergic neurons; indeed, degeneration outside the SN is relatively limited, and thus therapeutic efficacy, or lack thereof, in the nigrostriatal system does not necessarily translate to other areas and symptoms. Evaluation of therapeutics on non-motor symptoms of PD may therefore require targeted delivery of viral vectors to areas outside of the SN. Targeted delivery of AAVs encoding α-syn to the ventral tegmental area (VTA)[30] and the nucleus basalis[148] indicates that dopaminergic neurons of the VTA and cholinergic neurons of the nucleus basalis are also vulnerable to α-syn overexpression, although VTA neurons are less vulnerable than their SN counterparts. In an effort to implicate other areas outside of the SN, Ulusoy *et al.*[149] showed that vagal injection of AAV (wild-type human α-syn) produced thioflavin S-positive accumulations of α-syn in medullary nuclei and in axons as distant as forebrain areas.

Viral vector-based delivery models in non-human primates. Attempts have been made to translate the rodent viral vector models into NHPs (TABLE 1). A NHP model provides an opportunity to de-risk a clinical development programme, benefiting from: a more human-related anatomical organization and immune system than rodents; endogenous α-syn with the same sequence as human; behavioural measures that are closer to clinical end points; an ability to ensure target engagement (for example, using positron emission tomography (PET)); and definition of appropriate dosing moving forward. For example, in contrast to rodents, the brains of some primate species (including humans, rhesus macaques and cynomolgus macaques, but not marmosets) contain melanized neurons. Larger NHP species, including cynomolgus and rhesus macaques, have more translatable toxicology than do non-primate species and, unlike marmosets and rodents, which express T53 α-syn, express A53 α-syn, as do humans[150]. Despite the advantages of using macaques, AAV delivery of α-syn in NHPs was first achieved in marmosets; here, α-syn production by AAV2 delivery produced nigral dopamine neuron degeneration, dystrophic neurites and proteinase K-resistant aggregates within 1 year[151,152].

Despite the logistical challenges associated with working with larger NHPs, there would still be benefits from having a macaque-based model. An initial report, although using lentiviral delivery, demonstrated the feasibility of a rhesus macaque model. α-Syn pathology was observed at 8 weeks following viral vector delivery of human A53T into macaques (one 2 years old, one 8 years old and one 22 years old) and levels of pathology were higher in the older animals, although the results did not include comparison to a control group[153]. Recently, these findings were extended using a more attractive AAV vector (AAV1/2 expressing human A53T α-syn under a CMV or CBA promoter) in cynomolgus macaque. This study[154] used appropriate control groups and an optimized titre and surgical approach and, at 17 weeks following injection, demonstrated loss of ~50% SN dopaminergic neurons, ~72% loss of striatal dopamine, robust α-syn expression in >85% of surviving nigral neurons and transport of α-syn to the striatum (TABLE 1). To date, no behavioural deficits have been reported in these macaques, and thus the model should be considered as a model of α-synucleinopathy rather than of PD per se. Notwithstanding, for the first time, a robust macaque model of the molecular pathology of PD can help to evaluate disease-modifying therapies; moreover, the study provided measures of variability that can now inform power calculations to optimize design of NHP efficacy studies.

Models of spread

Following the descriptions of Braak *et al.* in 2002 (REF. 77), the hypothesis that α-syn spreads between interconnected anatomical areas, both within the brain and indeed from the olfactory bulb[155] or enteric nervous system[156] to the CNS, has become an attractive, if not compelling, way of modelling PD progression, prodromal PD and/or the premotor symptoms of PD. Although there are certain arguments against this hypothesis (BOX 3), models of α-syn spread that leads to degeneration have been developed. Notably, they currently represent models of a hypothesis rather than of a disease.

Cell-based systems initially defined potential mechanisms whereby α-syn might be transmitted between neurons[157–159]. Following this, elegant proof-of-concept studies in rodents showed transfer of α-syn overexpressed in host neurons — either endogenously (in transgenic mice[157,158]) or exogenously (through a viral vector[160,161]) — to grafted neurons. Thus, under experimental conditions, α-syn can move from one neuron into another *in vivo*. In moving towards an animal model that could be applied to drug development, unilateral, intrastriatal injection of mice with synthetic PFFs of presumably pathological α-syn, or with Lewy body-containing tissue from post-mortem human brain, initiated a cascading seeding event from the injection site[162,163]. This spread involved recruitment of endogenous α-syn and led to the widespread presence of pathological α-syn (as indicated by pSer129 immunoreactivity) throughout both hemispheres[162,163]. Considerable spreading of pathological α-syn was observed as early as 30 days and was increased further after 90 days[162,164,165]; however, cell loss[162] or changes in striatal dopamine levels were not observed until 180 days[166]. Cell phenotype has generally only been evaluated in the SN of these models, with the exception of cholinergic neurons of the dorsal motor nucleus of vagus being examined in models in which fibrils were delivered to the enteric nervous system[156]. More efforts could be made to understand the relationship between the presence of pSer129 α-syn reactivity in regions outside of the SN and degeneration therein.

Utility of models of spread. α-Syn spread models could have value in approaches interfering with α-syn accumulation and aggregation, but they might have most impact in evaluating therapies targeted at α-syn spread, extracellular α-syn or blocking of seeding. Consistent with this, an antibody specific for misfolded α-syn reduced Lewy pathology, SN dopamine cell loss and motor impairments assessed 180 days after inoculation of mice with α-syn PFFs[167]. Nonetheless, immunotherapies that are proposed to clear extracellular α-syn have shown efficacy in transgenic and in viral vector-based models, in which α-syn pathology has not been shown to spread, so this view may be oversimplistic.

Variability with the brain-extract models is a problem, as there is no clear means by which different extracts can be benchmarked and thus how a reliable source can be developed to provide consistent quality for a robust model. Moreover, the use of brain-extract models for drug development (at least that for commercial gain) may need to resolve issues relating to patient consent to tissue donation. The synthetic PFF approach, which does not require such consent and which can be standardized, may circumvent both of these issues.

Models of spread in non-human primates. The PFF or pathological brain-extract models can also be translated into NHPs, with all their advantages over rodents. This approach is also attractive as it could produce not only motor disability end points but also, as the pathology spreads through the brain, non-motor symptoms and related pathologies — for example, those related to loss and/or dysfunction of noradrenergic neurons of the locus coeruleus (which are involved in attention and sleep) or cholinergic neurons of the nucleus basalis (which are required for executive function and memory). Recasens *et al.*[168] showed in macaques that Lewy body-enriched midbrain extracts from PD brains could produce spreading of aggregated α-syn and degeneration of nigral dopamine neurons, although this degeneration took more than a year to become apparent (15–40% loss of nigral cells after 14 months).

Two proof-of-concept reports have recently emerged showing that injection of synthetic fibrils into striatal targets in NHPs produces Lewy pathology along the nigrostriatal tract of cynomolgus macaques after 12 months[169] and evidence of degeneration and spread of Lewy pathology outside of the basal ganglia of marmosets after 3 months[170]. These data support further development of primate PFF models to provide a model of α-syn spread in an anatomical system closer to that of humans.

Box 3 | **Controversies concerning the Braak hypothesis**

According to the Braak hypothesis, α-synuclein (α-syn) pathology spreads through the CNS in a caudal–rostral direction, and pathological α-syn is transmitted between anatomically connected systems. The Braak hypothesis forms the basis for the theoretical rationale behind the development of the preformed fibrils (PFF) α-syn-spreading models. However, spreading of α-syn pathology has not been definitively shown in Parkinson disease (PD), and the Braak hypothesis is solely based on 'snapshots' of patterns of degeneration in independent post-mortem PD cases at various times following diagnosis. Furthermore, the idea that α-syn transfer can occur under normal physiological conditions arguably challenges some basic tenets of cell biology[202]. A separate, alternative hypothesis is that selective vulnerability underlies the sequential emergence of pathology, as less-resistant regions become compromised earlier in the disease, whereas more-resistant regions benefit from cellular mechanisms of compensation for longer[203,204]. Thus, although α-syn can be transmitted between neurons under certain conditions, it is not clear whether the same occurs in humans.

Using models in drug development

We now share our personal perspectives on how, given the state of the art and the various advantages and limitations of the different model types (TABLE 2), mammalian models of α-synucleinopathy in PD might be most valuable in defining potential efficacy of a novel therapeutic approach. A flow chart illustrating the decision making for a potential small molecule therapeutic is presented in FIG. 2.

Before initiating any efficacy studies to validate a target, several issues should be considered. The major risk factor for developing PD is age. It is vital to ensure that the proposed target is present, and functioning with expected physiology, in age-appropriate human tissue,

Table 2 | **Advantages and limitations of different types of α-synucleinopathy models**

Type of model	Advantages	Limitations
Transgenic models	• High construct validity: based on genetic causes of PD in humans • Some face validity: occurrence of α-syn aggregates • Potential disease-modifying therapies have shown efficacy (for example, immunotherapies targeting α-syn[102,112,115]) • Model some peripheral α-syn pathology and non-motor symptoms • Can be established in laboratories from small founder laboratories or commercial suppliers • Transgenic mouse model expressing wild-type α-syn under the *Thy1* promoter has good face validity and is well studied	• Incomplete face validity: limited and inconsistent neurodegeneration in SN • Prolonged time course to develop pathology is not optimal for evaluation of potential therapeutics • Limited reliability and utility of motor phenotypes as outcome measures • Peripheral α-syn pathology and associated non-motor symptoms may not precede brain pathology • Predictive validity: not yet demonstrated • No well-validated NHP application yet
Viral vector delivery models	• High construct validity: based on the molecular pathology of PD, both genetic and sporadic • Strong face validity: models dopaminergic neuron degeneration, accumulation of α-syn aggregates and PD-like motor deficits • Develop phenotype in weeks to months, allowing medium throughput of candidate therapeutics • Can be applied across multiple species, including NHPs • Potential therapies have shown efficacy in rodent models[117,144–147] • Several new-generation AAV2-related rodent models are widely available and represent a viable platform to evaluate novel therapeutics	• Incomplete face validity: no non-motor phenotype, thus may require application of vectors outside the nigrostriatal system • Degeneration restricted to pathways emerging from site of focal delivery, typically the nigrostriatal pathway • Predictive validity: not yet demonstrated • Resource intensive, especially NHP models
α-Syn transmission models	• Construct validity: model trans-synaptic spread of α-syn • Some face validity: progressive accumulation of α-syn aggregates and degeneration beyond site of delivery • Potential to model α-syn pathology in multiple neurochemical systems and to produce non-motor symptoms	• Construct validity: dependent on the prion hypothesis • Time to develop pathology is long • Incomplete face validity: no PD-like behavioural motor deficits • Poorly characterized with respect to robustness of model in different laboratories and ability to show efficacy of potential therapeutics

α-Syn, α-synuclein; AAV2, adeno-associated virus 2; NHP, non-human primate; PD, Parkinson disease; SN, substantia nigra; *Thy1*, gene encoding thymus cell antigen 1.

ideally both in individuals with PD and in age-matched controls. It should then be demonstrated that the target is present and engageable with a defined treatment regimen in the model in which efficacy is to be evaluated.

We consider that at least two independent studies in rodent models are a minimum for progression to NHP evaluation. NHP evaluation valuably de-risks progression to clinical development because it addresses potential mechanistic differences between rodents and primates and, perhaps more importantly, ensures that appropriate target engagement can be achieved in the clinic and helps to define the therapeutic window; these points cannot be adequately achieved in rodents.

Irrespective of proposed mechanism of action of the therapeutic, our primary platform for evaluating efficacy remains the AAV α-syn rat. Our own experience has focused on AAV1/2 A53T α-syn, but AAV2/6 and AAV2/7 delivering mutant or wild-type human α-syn also provide reliable and reproducible pathology along with behavioural deficits within ~3–9 weeks. Once appropriate standard operating procedures have been developed and validated, the low variability of these end points within and between research groups and the low study duration are the major advantages of using AAV models as a primary rodent screen over available transgenic and PFF-based models. If the tool compounds are mouse specific (as might be the case for certain immune therapies), we would use an AAV α-syn mouse model as the primary model of evaluation[171].

After a first demonstration of efficacy in an AAV-based rodent model, we would seek at least one additional, independent confirmatory study in rodents. At this stage, one could add controls to demonstrate that the treatment does not interfere with AAV mechanics, to determine that there is an effect using wild-type α-syn and to assess efficacy when treatment is commenced at different times in the model (FIG. 2). Demonstration of efficacy in both studies would be a prerequisite for progression of the development plan.

NHP models can enable valuable assessment of compound efficacy, safety and target engagement in the clinical situation. Our decision to progress to NHP evaluation is driven directly by the efficacy in the second AAV rat study. In parallel with initiating the NHP study, we also consider evaluating efficacy in a model with a different pathology-generating approach, perhaps with a longer

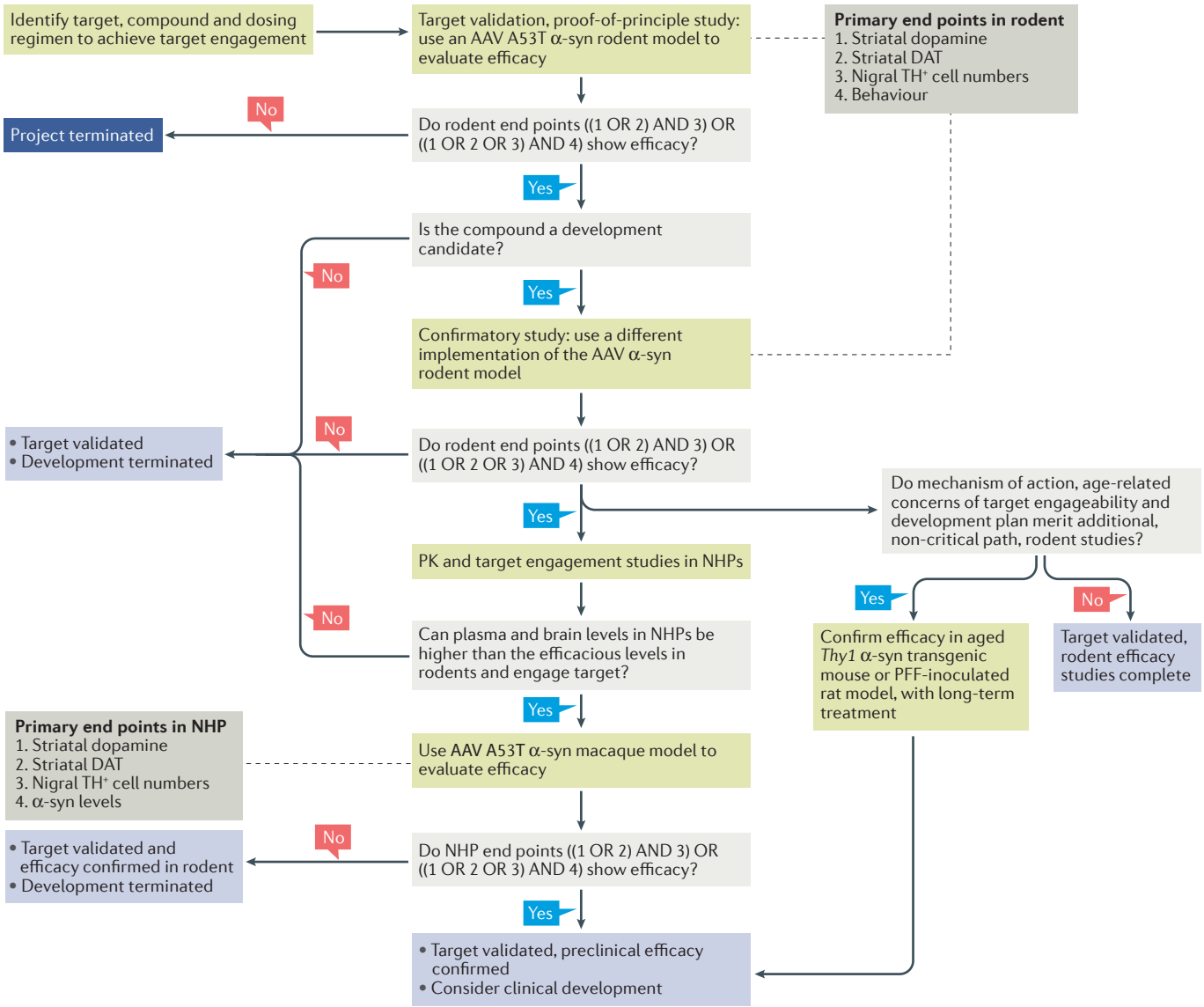


Figure 2 | **Identification of potential disease-modifying action: a logic flow chart.** This figure describes our preferred development and decision-making pathway from the identification of a potential target, via evaluation and to a strong, informed decision as to whether to initiate clinical development. If feasible, we ensure before efficacy assessment that the target is present in people with Parkinson disease (PD) and, if appropriate, that signalling downstream of the target is intact in PD. This is especially important if it is possible that age or accumulation of α-synuclein (α-syn) might affect target function. We then ensure target engagement in the system in which the efficacy of the candidate therapeutic will be evaluated, typically using a combination of pharmacokinetics (PK)–pharmacodynamics and *ex vivo* measures of engagement. Our next step is to validate the target, providing proof of principle typically in an adeno-associated virus (AAV) A53T α-syn rodent model. We consider a target validated if we demonstrate target engagement and attenuate deficits in one presynaptic measure of striatal dopaminergic function (such as levels of dopamine or dopamine transporter (DAT)) and in one measure of nigral dopamine neurons (for example, stereological counting of tyrosine hydroxylase (TH)-positive (TH^+) cell number) or if we provide substantial benefit against a dopaminergic deficit and recovery at a behavioural level. If the aim of the approach is to promote α-syn clearance, then we consider the target validated if we can reduce α-syn load and attenuate deficits in one or more of the primary dopaminergic markers. If the approach being evaluated can be developed as a therapeutic, we would confirm the robustness of the target validation in a further rodent study. For reasons outlined in the main text, we typically prefer to use an additional AAV-based rodent model, but perhaps with a different species, a different AAV serotype or a vector that delivers wild-type α-syn, rather than A53T α-syn. This confirmatory study should also include suitable control groups to ensure that the candidate therapeutic does not directly interact with the vector, the transgene or the transgene promoter. At this point, one could also explore commencing treatments at different times, before and after delivery of AAV, to understand the potential to prevent, as well as clear, α-syn pathology. If efficacy is confirmed, we would move to the final preclinical evaluation stage. The core of this stage is an efficacy study in a non-human primate (NHP) model, produced by AAV α-syn delivery in the macaque. Dosing regimens are informed by target engagement studies, as in rodents, but ideally also incorporate imaging measures, which can help later translation. In some instances, we would also incorporate a parallel, additional rodent study that is not necessarily on the critical path of decision making but that could provide additional information on, for example, mechanism of action or the effect of age. With respect to the latter, for example, the aged transgenic mouse expressing α-syn under the control of the thymus cell antigen 1 (*Thy1*) reporter can be particularly useful. In the NHP efficacy test, as in the rodent, we define a priori the conditions that determine whether an approach is successful and worthy of considering for clinical development. PFF, preformed fibril.

time to readout, or with non-motor symptoms or in aged animals. The value of this depends on the mechanism of action, whether there is an intention to develop a clinical programme targeting non-motor symptoms or if the studied target is thought to be particularly affected by age. The choice could be, for example, transgenic mice expressing α-syn under the control of the *Thy1* promoter (which are excellent for demonstrating α-syn clearing and some non-motor symptoms) or the rat PFF model (which can evaluate protection or α-syn clearing in non-dopaminergic systems and directly addresses interactions with α-syn spread, should that occur). Owing to variability in the sources of human brain extracts, we do not feel that the Lewy body-derived extract models are currently realistic alternatives to PFFs for therapeutic development. This additional, confirmatory, long-term rodent model can be completed alongside initiation of the NHP evaluation and enhances understanding of the mechanism of action alongside the readouts from the NHP study, thus informing on whether to progress to clinical development. AAV vector-based approaches, with well-characterized AAV1/2 models available in rodent and NHP, provide a package of data to inform clinical translation. Thus, for now, our NHP evaluation would be based on bilateral SN delivery of AAV1/2 encoding A53T α-syn. Our primary end points would relate to pathology, specifically degeneration of the nigrostriatal pathway and Lewy-like pathology. AAV or transgenic models in NHP that model pathology in non-nigral regions are currently not sufficiently developed to be useful in drug-development programmes. NHP models based on Lewy body-derived extracts, although valuable scientifically in understanding the plausibility of the prion-like spread hypothesis, do not currently have a place in our drug-development scheme.

Future directions

At present, the α-synucleinopathy models available that have qualities appropriate for drug development are limited. In FIG. 2, we propose how the currently available models can be used in PD drug discovery. As we broaden this range of models, especially as new transgenic and fibril-based models are optimized, we envisage a more sophisticated flow of decision making that would be driven primarily by consideration of the proposed mechanism of action.

We anticipate that developments will continue across all three of the major model types identified above and that continued support will be needed to further understand the biochemistry and structural characterization of the aggregates produced therein. Given previous failures with compounds emerging from toxin-based models, the target engagement of all potentially disease-modifying therapies needs to be confirmed on a background of α-syn pathology. The models currently available, both rodent and primate, are fit for this purpose.

In the near term, the viral vector-based NHP models that are becoming available have potential to identify therapies for clinical evaluation. This will either validate or invalidate the models as predictors of phase II efficacy and provide feedback that will help to refine design and to interpret the translatability of findings. In rodents, we expect an increasing use of focal injection of vectors outside the SN to model pathology underlying non-motor symptoms.

In the medium term, it is not clear how robust or variable the PFF or brain-extract models will be once applied widely in multiple laboratories or how they will translate to other species. Moreover, the attraction of the transmission models largely relies on the still controversial prion hypothesis and, should this fall out of favour and/or the models produced not be widely reproducible, the use of this approach could contract into niche areas of research, similar to the use of rotenone in modelling mitochondrial toxicity.

Experience with rodents suggests that long-term investment in transgenic NHP models is both necessary and, given recent data, feasible. However, building colonies to provide research needs will probably take a decade or longer. Newly available technology (such as CRISPR) will benefit transgenic modelling in NHPs. Transgenic technology provides an opportunity to produce a PD macaque model with a developing phenotype and pathology that model the progression of clinical PD and will be extremely valuable in producing cohorts of animals with which to evaluate potential therapies at various disease stages and for different symptomatologies.

We propose that, for at least the next 5 years, the development of disease-modifying agents for PD should require a preclinical demonstration of controlling α-syn-related pathology or deficits arising from such. As approaches transition from the models reviewed here into clinical development, the predictive value of the models discussed will become clearer and, irrespective of clinical outcomes, their use in therapeutic development will be refined and improved.

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