ORGANIC NETWORK SYNTHESIS

α-Synuclein Templating and the Molecular Biology of Synucleinopathy Propagation: A Critical Evaluation of the Lee–Trojanowski Research Program

Lee · Trojanowski

Critical Review — Editor Protocol Applied

Prepared for Dr. James Truchard and Benjamin Gustafsson
Drafted by Claude-Opus-4.7 under the ONS Methodology
AdultCognitiveDisease.com · May 2026

α-Synuclein Templating and the Molecular Biology of Synucleinopathy Propagation: A Critical Evaluation of the Lee–Trojanowski Research Program at the University of Pennsylvania Center for Neurodegenerative Disease Research

Abstract

For nearly three decades, the laboratory of Virginia M.-Y. Lee and the late John Q. Trojanowski at the University of Pennsylvania Center for Neurodegenerative Disease Research (CNDR) has occupied the central position in α-synuclein biology, supplying both the molecular tools and the conceptual architecture by which the synucleinopathies — Parkinson's disease (PD), dementia with Lewy bodies (DLB), and multiple system atrophy (MSA) — are now understood as templated proteinopathies. This thesis offers a critical evaluation of four interlocking contributions that define the program: (1) the 1997–1998 identification of α-synuclein as the principal fibrillar component of Lewy bodies and Lewy neurites,[1,2] a finding that, together with the Polymeropoulos identification of *SNCA* point mutations in autosomal-dominant PD,[3] unified PD and DLB under the single banner of "α-synucleinopathy"; (2) the development of recombinant pre-formed fibrils (PFFs) as a reproducible tool for inducing Lewy-like pathology in cultured neurons[4] and wild-type mouse brain,[5,6] displacing the prior generation of transgenic overexpression models; (3) the experimental demonstration that α-synuclein pathology propagates trans-synaptically along defined anatomical projections, recapitulating in mice the stereotyped staging Heiko Braak had described in human autopsy material;[7] and (4) the establishment that α-synuclein exists as distinct strains, with the PD/DLB strain and the MSA strain exhibiting different conformational signatures, different cellular tropism (neuronal vs oligodendroglial), and different transmission kinetics.[8,9]

Read together with the prion-like paradigm of Stanley Prusiner[10] and the seeded-transmission program of Walker and Jucker,[11] the Lee–Trojanowski body of work supplies the synucleinopathy-specific instantiation of templated misfolding that completes the molecular framework on the α-synuclein axis. The PFF model has become the field's workhorse for testing therapeutics; the strain framework has reframed MSA as a categorically distinct disorder rather than an aggressive PD variant; and the trans-synaptic spread paradigm provides the disease-progression logic that immunotherapeutic and small-molecule strategies must now engage. Trojanowski's death in February 2022 closed the founding partnership; the laboratory continues under Lee's direction.

> **What remains uncertain:** *Whether the strain distinction between PD/DLB and MSA reflects a stable two-strain dichotomy or a continuous conformational landscape from which sub-strains emerge as a function of cellular environment; and whether the earliest seeding site of synucleinopathy is the olfactory bulb, the enteric nervous system, or the locus coeruleus — a question the Lee–Trojanowski tools have illuminated but not resolved.*

Introduction

Prior to the late 1990s, α-synuclein occupied an obscure position in molecular neuroscience. The protein had been identified in 1988 by Maroteaux and colleagues as a synaptic-vesicle-associated protein of unknown function in the electric ray *Torpedo californica*,[12] and a homologue had been cloned from human brain shortly thereafter. Its physiological role was undefined; its disease relevance was unsuspected. The dominant molecular framework for Parkinson's disease in this period was almost entirely neurochemical: the loss of nigrostriatal dopaminergic neurons, the depletion of striatal dopamine, the responsiveness of motor symptoms to L-DOPA, and the susceptibility of dopaminergic neurons to the mitochondrial complex-I inhibitor MPTP. The eosinophilic cytoplasmic inclusions Friedrich Lewy had described in 1912 — Lewy bodies — were a defining pathological feature, but their molecular composition was unknown. Most investigators presumed they contained neurofilament protein, ubiquitin, or some combination of cytoskeletal debris.

Two papers in 1997 transformed the field within a calendar year. In June, Polymeropoulos and colleagues at the National Institutes of Health reported in *Science* the identification of a single amino-acid substitution — A53T — in the *SNCA* gene encoding α-synuclein, segregating with autosomal-dominant Parkinson's disease in an Italian-American kindred (the Contursi family) and three Greek kindreds.[3] The genetic finding was decisive: α-synuclein, however obscure its physiological role, could cause Parkinson's disease when mutated. In August 1997, Maria Grazia Spillantini, working in Michel Goedert's laboratory in Cambridge, with collaborators including Lee and Trojanowski at Penn, demonstrated in a *Nature* brief communication that α-synuclein is the major fibrillar component of Lewy bodies and Lewy neurites in sporadic Parkinson's disease and dementia with Lewy bodies.[1] The 1998 follow-up papers — Spillantini, Crowther, Jakes, Hasegawa, Goedert in *PNAS*[2] and the parallel work from Lee, Trojanowski, and colleagues — established the biochemistry: α-synuclein in Lewy bodies is fibrillar, ubiquitinated, and post-translationally modified, with phosphorylation at serine-129 emerging as a near-universal marker of pathological α-synuclein.

The unification was immediate and consequential. PD and DLB, previously categorized as clinically distinct disorders, were now revealed as variants of a single underlying molecular

pathology: α-synucleinopathy. Multiple system atrophy (MSA), in which the inclusions are predominantly oligodendroglial rather than neuronal (glial cytoplasmic inclusions, GCIs), was rapidly added to the family when its inclusions were shown to contain α-synuclein as well.[13] By the close of the 1990s, the conceptual landscape had been rewritten: three clinically and pathologically distinct disorders shared a single misfolded protein.

The next conceptual leap took a decade. In 2008, two independent groups — Kordower, Brundin and colleagues, and Olanow and colleagues — reported that fetal mesencephalic neurons transplanted into the striata of Parkinson's patients more than a decade earlier had themselves acquired Lewy-body pathology at autopsy.[14,15] The grafted neurons had been genetically and developmentally young; their accumulation of α-synuclein pathology implied that some pathogenic species had moved from the host brain into the graft. In 2009, Desplats and Masliah demonstrated experimentally that α-synuclein can be transmitted from neuron to neuron in cell culture and in transgenic mice.[16] The prion-like question was now openly on the table for α-synuclein. Lee's PFF program resolved it. The remainder of this thesis traces the program's logic — historical, methodological, mechanistic, and therapeutic — and situates it within the broader templated-misfolding paradigm that has come to dominate the field.

Literature Review and Theoretical Positioning

The historiography of α-synuclein in neurodegeneration unfolds in four distinguishable phases. The first phase, spanning roughly 1988 to 1996, is one of molecular obscurity. The protein had been characterized in *Torpedo*, in zebra finch song nuclei (where it was implicated in synaptic plasticity associated with vocal learning), and in human brain.[12] Its presynaptic localization and its association with synaptic vesicles were established. Its function, however, remained speculative — variously implicated in vesicle clustering, SNARE-complex assembly, dopamine release, and lipid binding. No disease association had been documented.

The second phase, 1997–1998, is the foundational disease-association phase. The Polymeropoulos *Science* paper identifying the A53T mutation in autosomal-dominant PD[3] arrived first; subsequent papers identified additional point mutations (A30P, E46K, H50Q, G51D) and *SNCA* locus multiplications.[17,18] The Spillantini–Goedert *Nature* brief communication identifying α-synuclein in Lewy bodies[1] arrived in August 1997; the more comprehensive 1998 *PNAS* paper followed.[2] Lee and Trojanowski's parallel biochemical work — purifying α-synuclein from human Lewy-body-bearing brain and demonstrating its fibrillar character, its ubiquitination, and its phosphorylation at Ser129 — supplied the molecular foundation on which the entire subsequent program rests. Wakabayashi and colleagues[13] rapidly extended

the finding to the glial cytoplasmic inclusions of MSA, completing the unification of the synucleinopathies.

The third phase, 2003–2009, is the staging and propagation phase. Heiko Braak and colleagues published in 2003 a staging system for Lewy pathology in PD that proposed a stereotyped caudo-rostral spread: from the dorsal motor nucleus of the vagus and the olfactory bulb, ascending through the brainstem, then through the limbic system, and finally to the neocortex.[7] The Braak staging implied — though did not demonstrate — that α-synuclein pathology propagates through the brain along defined anatomical pathways. The 2008 Kordower–Brundin and Olanow grafting papers[14,15] supplied the first direct evidence in human tissue that something had moved from host to graft. The 2009 Desplats–Masliah *PNAS* paper[16] demonstrated cell-to-cell transmission experimentally. By the close of 2009, the prion-like hypothesis was a serious working model awaiting decisive experimental confirmation.

The fourth phase, 2011 to the present, is the mechanistic phase, dominated by the Lee–Trojanowski program. The 2011 Volpicelli-Daley *Neuron* paper introduced the PFF model in cultured neurons,[4] demonstrating that exogenous recombinant α-synuclein fibrils could seed Lewy-like pathology in genetically unmodified primary neurons. The 2012 Luk *Science*[5] and *Journal of Experimental Medicine*[6] papers extended the model to wild-type mouse brain via stereotaxic intracerebral injection. Pathology appeared at the injection site, then in anatomically connected regions, then in increasingly distal cortical and brainstem nuclei, recapitulating the Braak staging in a tractable laboratory model. The 2018 Peng *Nature* paper distinguished PD/DLB and MSA strains experimentally,[8] and the 2020 Schweighauser cryo-EM structures[9] resolved the structural basis at near-atomic resolution. The Kim–Mao 2019 *Neuron* paper extended the model to gut-to-brain propagation via vagal injection,[19] consistent with the body-first hypothesis of Borghammer and others.[20]

The Lee–Trojanowski program is thus the central spine of the third and fourth phases. The molecular biology of α-synuclein in disease — what the pathogenic species is, how it propagates, how it varies across disease entities — is largely the biology this laboratory built. Critical engagement with the program requires both an appreciation of its empirical dominance and an honest reckoning with the questions it has illuminated without resolving.

Analytical Framework and Methodology

This analysis adopts the same integrative methodology applied across the convergent-synaptic-collapse paper series: a critical synthesis of primary peer-reviewed literature, evaluation of experimental rigor in light of model-system limitations, and explicit positioning of the Lee–Trojanowski findings within the broader prion-like framework supplied by Prusiner[10]

and Walker–Jucker.[11] Four methodological pillars of the Lee–Trojanowski program warrant specific attention because they determine both the program's reach and its boundaries.

The first pillar is recombinant α-synuclein purification. Production of high-purity, endotoxin-controlled recombinant human α-synuclein from *Escherichia coli*, followed by buffer exchange into physiological conditions, supplies the monomeric starting material from which fibrils are subsequently generated. The protocol is reproducible across laboratories — a critical property for a model intended to serve as the field's standard. Lee's group has published detailed protocols in *Nature Protocols* ensuring methodological transparency.[21]

The second pillar is in vitro fibrillization. Monomeric α-synuclein, incubated at high concentration (typically 5 mg/mL) with continuous agitation at 37°C, spontaneously assembles into amyloid fibrils over the course of several days. The resulting fibrils are characterized by thioflavin-T fluorescence, by transmission electron microscopy demonstrating the canonical 10-nm filament morphology, and by sedimentation properties. The mature fibrils are then sonicated to generate the smaller seeding-competent fragments that constitute the PFFs — typically 50–100 nm in length, with the higher fragmentation enabling more efficient cellular uptake and intracellular nucleation.[21]

The third pillar is the cell-culture and in vivo seeding assay. In the Volpicelli-Daley protocol,[4] primary mouse hippocampal neurons cultured for approximately one week are exposed to PFFs in the medium. Within seven to fourteen days, the exogenous fibrils template the misfolding of endogenous mouse α-synuclein, generating phospho-Ser129-positive, insoluble, ubiquitinated inclusions that morphologically and biochemically recapitulate Lewy pathology. Critically, the inclusions are composed predominantly of endogenous protein, not the exogenous seed — the seed is catalytic, not stoichiometric. In the in vivo Luk protocol,[5] 5 µg of sonicated PFFs are injected stereotaxically into the dorsal striatum of wild-type adult C57BL/6 mice. Pathology appears at the injection site by 30 days, in interconnected cortical regions by 90 days, and in the substantia nigra (the canonical Parkinsonian target) by 180 days, accompanied by progressive dopaminergic neuron loss and motor deficits.

The fourth pillar is immunohistochemistry for phospho-Ser129 α-synuclein (pS129). Because Ser129 phosphorylation is a near-universal feature of pathological α-synuclein but is rare on physiological monomeric α-synuclein in healthy brain, pS129 antibodies (notably the EP1536Y rabbit monoclonal and the 81A mouse monoclonal) supply a robust, pathology-specific stain that permits time-course analysis of pathology propagation across mouse brain. The temporal cascade — injection-site pathology preceding distal pathology by weeks to months — is the empirical foundation of the trans-synaptic spread argument.

The methodology has limitations the program has not fully escaped. The PFFs used as seeds are recombinant and lack the post-translational modifications and lipid associations of fibrils extracted from human brain. The cellular environment of a mouse cortical neuron is not

identical to that of a human substantia-nigra dopaminergic neuron. The temporal scale of mouse experiments (months) is several orders of magnitude shorter than human disease progression (decades). These limitations matter for therapeutic translation but do not undermine the core mechanistic findings the program has established.

Chapter 1: α-Synuclein as the Lewy Body Protein

The first foundational contribution of the Lee–Trojanowski program — though one shared with the Goedert–Spillantini laboratory in Cambridge — was the demonstration in 1997–1998 that α-synuclein is the principal molecular constituent of Lewy bodies and Lewy neurites in Parkinson's disease and dementia with Lewy bodies. The finding completed the genetic-pathological loop opened by the Polymeropoulos identification of the A53T mutation, established a unified molecular framework for the synucleinopathies, and set the agenda for the next quarter-century of the field.

1.1 The Polymeropoulos Genetic Foundation

The June 1997 *Science* paper from Polymeropoulos and colleagues[3] reported genetic linkage of autosomal-dominant Parkinson's disease in the Contursi kindred to chromosome 4q21–q23, followed by identification of an alanine-to-threonine substitution at codon 53 (A53T) in the *SNCA* gene encoding α-synuclein. The mutation segregated with disease in the Italian kindred and in three independent Greek kindreds. The finding was transformative for two reasons. First, it identified a single protein whose disturbed sequence could cause a phenotypically classical Parkinson's disease, anchoring the entire syndrome to a specific molecular target. Second, it implicated a protein that had been hiding in plain sight — abundant, presynaptic, evolutionarily conserved, and previously assumed to be physiologically innocuous.

The Polymeropoulos paper alone, however, addressed only a tiny fraction of PD cases (familial autosomal-dominant disease accounts for under 10 percent of PD; the A53T mutation accounts for a vanishingly small fraction of that). The pivot from a rare-mutation finding to a sporadic-disease unifier required demonstrating that α-synuclein is not only mutated in rare familial PD but is the molecular constituent of Lewy pathology in the overwhelmingly more common sporadic disease.

1.2 The 1997–1998 Identification of α-Synuclein in Lewy Bodies

The August 1997 *Nature* brief communication from Spillantini, Schmidt, Lee, Trojanowski, Jakes, and Goedert[1] reported immunohistochemical evidence that α-synuclein is present in Lewy bodies and Lewy neurites in sporadic PD and DLB. The 1998 follow-up paper in *PNAS*[2]

extended the finding biochemically: α-synuclein extracted from PD brain was fibrillar, insoluble in sarkosyl, and recovered in detergent-resistant fractions consistent with amyloid assembly. The fibrils displayed the canonical cross-β architecture, with mean filament widths of approximately 10 nm and an axial repeat consistent with the parallel in-register β-sheet assembly subsequently confirmed by cryo-EM.

Lee and Trojanowski's parallel biochemical work, drawing on the CNDR brain bank's accumulated tissue resources, established the post-translational modifications of pathological α-synuclein. Phosphorylation at serine-129 was identified as a near-universal feature of α-synuclein in Lewy bodies, while monomeric soluble α-synuclein in healthy brain is largely unphosphorylated.[22] Ubiquitination of pathological α-synuclein was demonstrated, consistent with its recognition by the cellular proteostatic machinery — and with the failure of that machinery to clear it. C-terminal truncations were identified as enhancing aggregation propensity in vitro and as enriched in pathological inclusions.

1.3 Unification of the Synucleinopathies

The Wakabayashi 1998 paper[13] rapidly extended the α-synuclein identification to the glial cytoplasmic inclusions (GCIs) of multiple system atrophy, in which oligodendrocytes — not neurons — harbor the principal pathology. The finding was conceptually significant: it demonstrated that a single misfolded protein could produce different disease entities depending on the cellular compartment in which it accumulated. PD and DLB became "neuronal synucleinopathies"; MSA became an "oligodendroglial synucleinopathy." The framework also accommodated the lesser-known synuclein-containing inclusions in the axons of patients with pure autonomic failure.

This unification had immediate diagnostic consequences. Phospho-Ser129 immunohistochemistry, developed largely on the foundation of Lee's biochemical work, became the gold-standard neuropathological tool for synuclein pathology, displacing the older silver-impregnation and ubiquitin stains. The DLB consortium criteria, the McKeith DLB diagnostic guidelines, and the MSA neuropathological criteria all came to depend on α-synuclein-based pathology assessment. The conceptual unification under "α-synucleinopathy" reframed clinical trial design: a drug targeting α-synuclein could in principle benefit patients across PD, DLB, and MSA, expanding both the therapeutic target population and the regulatory pathway for sponsor companies.

The historiographical significance of the 1997–1998 cluster cannot be overstated. Within twelve months, a previously obscure presynaptic protein had become the central molecular target of three previously distinct neurodegenerative disorders. The Lee–Trojanowski laboratory, by virtue of both its analytical expertise and its access through CNDR to extensive au-

topsy material, was positioned at the center of this transformation — and remained at the center as the field shifted from identification to mechanism over the following decade.

Chapter 2: Pre-Formed Fibrils — A Reproducible Model of Synucleinopathy

The 1997–1998 identification of α-synuclein as the Lewy body protein created a methodological problem the field would struggle with for the following decade. The pathological species could be visualized and biochemically characterized in postmortem human tissue, but the mechanism of pathogenesis required a tractable laboratory model. The initial response — α-synuclein transgenic mice overexpressing wild-type or mutant human α-synuclein — produced models that exhibited some features of synucleinopathy but suffered from major limitations: pathology was dependent on supraphysiological expression levels, the cellular distribution of pathology often did not match human disease, and the artificial transgene context complicated interpretation. The field needed a method to induce synucleinopathy in genetically unaltered neurons, on a tractable timescale, with morphological and biochemical fidelity to human disease. The Lee laboratory supplied it.

2.1 The Volpicelli-Daley 2011 Cell-Culture Model

The landmark Volpicelli-Daley *Neuron* paper of 2011[4] introduced the cell-culture PFF protocol that has since become a global standard. The methodology was conceptually simple but experimentally consequential: recombinant human α-synuclein was purified, fibrillized in vitro under agitation, sonicated to generate seeding-competent fragments (the "pre-formed fibrils" or PFFs), and added directly to the medium of cultured primary mouse hippocampal neurons. Within seven days, the exogenous fibrils were demonstrably internalized; by 14 days, the recipient neurons exhibited phospho-Ser129-positive, sarkosyl-insoluble, ubiquitinated inclusions; by 21 days, the inclusions exhibited the morphology of mature Lewy bodies and Lewy neurites.

Three properties of the model were decisive. First, the induced pathology was composed predominantly of endogenous neuronal α-synuclein, demonstrated by experiments in which fluorescently tagged PFFs were used to seed cultures of neurons expressing untagged endogenous protein — the resulting inclusions were untagged, confirming that the seed had templated the misfolding of native cellular protein rather than simply aggregating with it. This is the molecular definition of templated misfolding: a catalytic conversion of a native molecule into the pathological conformation. Second, the pathology recapitulated the post-translational signature of human Lewy pathology — phospho-Ser129, ubiquitination, C-terminal truncation, and resistance to non-ionic detergents. Third, the pathology produced

demonstrable functional consequences: progressive synaptic dysfunction, loss of synaptic proteins (synapsin, SNAP-25), and eventual neuronal death over a time course of weeks.

The methodological revolution this represented for the synucleinopathy field is difficult to overstate. Prior to 2011, induction of synucleinopathy in primary neurons had required transgenic overexpression of disease-associated *SNCA* variants. The PFF model achieved comparable or superior pathology in wild-type cells, on a defined timescale, with a single exogenous reagent that could be standardized across laboratories. By 2015, the protocol had been adopted in dozens of laboratories worldwide and had become the de facto cell-culture standard for synucleinopathy research.

2.2 The Luk 2012 In Vivo Translation

The translation of the cell-culture model to intact mouse brain followed within a year. The Luk *Science* paper of 2012, *"Pathological α-synuclein transmission initiates Parkinson-like neurodegeneration in non-transgenic mice,"*[5] reported that stereotaxic injection of 5 µg of sonicated PFFs into the dorsal striatum of wild-type adult C57BL/6 mice induced progressive synucleinopathy with a time course and anatomical distribution consistent with human Parkinson's disease. The companion Luk paper in the *Journal of Experimental Medicine*[6] extended the model to intracerebral inoculation in mice overexpressing the A53T human α-synuclein transgene, producing rapidly progressive synucleinopathy and demonstrating that the seeding-competent species was active in vivo across multiple genetic backgrounds.

The temporal cascade observed in the wild-type mouse model is the empirical backbone of the trans-synaptic spread argument. Pathology appeared first at the injection site within 30 days, in cortical regions reciprocally connected to the striatum (motor cortex, insular cortex) by 60–90 days, in the substantia nigra by 90–180 days, and in increasingly distal regions over the subsequent six to twelve months. Critically, dopaminergic neurons in the substantia nigra exhibited progressive loss accompanied by motor deficits on rotarod and beam-walking assessments — phenotypes recapitulating the cardinal features of human PD in a wild-type, non-overexpression model.

The wild-type genetic background of the model was the key methodological breakthrough. Prior mouse models of synucleinopathy had universally required transgenic overexpression — typically with the human *SNCA* transgene driven by tyrosine hydroxylase, Thy1, or prion promoters at expression levels several-fold above physiological. The Luk model produced Lewy-like pathology and dopaminergic neurodegeneration in mice whose only α-synuclein was endogenous mouse protein at physiological levels. The pathological cascade was initiated by a defined exogenous seed and propagated through endogenous protein, providing the cleanest possible experimental separation of seeding from substrate.

2.3 Subsequent Extensions and Refinements

The years following 2012 saw extensive refinement and extension of the PFF model. Volpicelli-Daley and colleagues quantified the structural requirements for seeding competence, demonstrating that sonication-generated 50–100 nm fragments are substantially more seeding-competent than longer fibrils.[23] Luk and colleagues demonstrated that human-derived α-synuclein from PD brain extracts could seed pathology in mice with kinetics distinct from those of recombinant PFFs, suggesting that the human pathological species carries conformational information not fully recapitulated by in vitro-fibrillized recombinant protein.[24] Henderson and Lee demonstrated that PFFs injected into the olfactory bulb produced rostro-caudal propagation along olfactory projections, complementing the Rey–Brundin work and supplying experimental support for the olfactory-bulb-first variant of the Braak staging hypothesis.[25]

The PFF model has limitations the field has acknowledged but not fully resolved. The kinetics of induced pathology are several orders of magnitude faster than those of human disease (months versus decades), raising questions about whether the seeding species and the disease species are biochemically identical. The recombinant origin of the seed lacks the post-translational modifications and lipid associations of brain-derived pathological species. Cellular tropism in mice does not fully recapitulate the regional vulnerability of human PD — for instance, locus coeruleus involvement, which is among the earliest in human disease, is variable in PFF-injected mice. These limitations are real but have not prevented the model from becoming the indispensable workhorse of synucleinopathy research.

> **What remains uncertain:** *Whether recombinant in-vitro-generated PFFs constitute the same conformational species as the pathological α-synuclein that propagates in human disease, or whether they represent a related but biochemically distinct family of fibrillar conformers. The 2018 strain papers and the 2020 cryo-EM structures suggest that brain-derived and recombinant fibrils may exhibit distinguishable folds, raising the prospect that the field's standard model engages only a subset of the conformational landscape relevant to human disease.*

Chapter 3: Trans-Synaptic Spread Along Anatomical Projections

The PFF model supplied the methodological substrate for the third major contribution of the Lee–Trojanowski program: the experimental demonstration that pathological α-synuclein propagates through the brain trans-synaptically, along defined anatomical projections, in a stereotyped pattern that recapitulates the Braak staging derived from human autopsy material. This was the program's decisive contribution to the prion-like paradigm: where Prusiner[10] and Walker–Jucker[11] had established the conceptual framework and the experimental seed-

ing architecture, the Lee laboratory demonstrated that for α-synuclein specifically, the propagation respects the brain's own wiring diagram.

3.1 The 2012 Luk Demonstration of Connectivity-Respecting Spread

The 2012 Luk *Science* paper[5] included the first decisive demonstration that PFF-induced pathology spreads along anatomical projections rather than diffusing isotropically from the injection site. Following dorsal striatum injection, pathology appeared in cortical regions known to project to the striatum (motor cortex, somatosensory cortex, frontal association cortex) on a timeline consistent with retrograde transport, and in substantia nigra dopaminergic neurons via the nigrostriatal projection. Critically, regions *not* anatomically connected to the injection site remained pathology-free even at long time points, ruling out passive diffusion as the propagation mechanism.

The pattern of spread reproduced features of Braak's clinical PD staging. The Braak hypothesis, articulated in 2003,[7] proposed that Lewy pathology in idiopathic PD originates in the dorsal motor nucleus of the vagus and the olfactory bulb, then ascends through the lower brainstem (locus coeruleus), through the midbrain (substantia nigra), into the limbic system, and finally into the neocortex. The PFF mouse model permitted experimental testing of this staging hypothesis by varying the injection site. Injection into the dorsal motor nucleus of the vagus produced ascending brainstem pathology; injection into the olfactory bulb produced rostro-caudal pathology along olfactory projections; injection into the striatum produced predominantly cortical and nigral pathology. The pattern of distal pathology in each case respected the connectivity of the injection site, supporting a model in which pathological α-synuclein moves between neurons along synaptic contacts.

3.2 The Rey–Brundin Olfactory Bulb Program

The complementary program of Patrik Brundin, working at the Van Andel Institute, focused on the olfactory bulb as a candidate originating site of synucleinopathy. The Rey 2013 paper[26] demonstrated that PFFs injected into the olfactory bulb of wild-type mice produced rostro-caudal propagation of pS129-positive pathology along defined olfactory projections — anterior olfactory nucleus, piriform cortex, olfactory tubercle, and eventually amygdala and entorhinal cortex. The pattern was consistent with the Braak hypothesis and provided the experimental complement to the Lee laboratory's striatal-injection model.

The Brundin program also contributed crucial early evidence for cell-to-cell transmission in human tissue. The Kordower–Brundin and Olanow 2008 reports of α-synuclein pathology in fetal mesencephalic transplants[14,15] remain the most decisive in vivo human evidence for prion-like α-synuclein transmission. The grafted neurons had been embryonic at the time of transplantation and had aged for ten to fourteen years in the host brain before postmortem

analysis. Their accumulation of α-synuclein pathology, while host neurons of the same age in adjacent striatum were also pathological, supplied the foundational human observation that the PFF mouse model subsequently reproduced experimentally.

3.3 The Kim–Mao 2019 Gut-to-Brain Extension

The 2019 Kim, Kwon, Kam *Neuron* paper from the Dawson and Ko laboratories at Johns Hopkins,[19] using Lee-developed PFF reagents, extended the propagation paradigm to the enteric nervous system. The investigators injected α-synuclein PFFs into the muscularis layer of the duodenum and pylorus of wild-type mice. Over the following months, phospho-Ser129-positive pathology appeared first in the enteric ganglia, then in the dorsal motor nucleus of the vagus, then in the locus coeruleus, then in the substantia nigra, and eventually in the cortex. The temporal cascade respected the anatomy of the vagal projection: vagotomy performed prior to gut PFF injection abolished the propagation to brain.

The finding was conceptually significant because it experimentally validated the "body-first" hypothesis of PD pathogenesis, associated most prominently with Per Borghammer's clinical work.[20] Borghammer had proposed, on the basis of imaging and clinical observations, that a substantial subset of PD cases originate not in the brain but in the enteric or autonomic nervous system, with vagal ascent to brainstem and subsequent cortical involvement. The Kim paper provided a mechanistic model: pathological α-synuclein in the gut can, in principle, reach the brain via the vagus, and the seeded propagation respects vagal anatomy.

3.4 Neuroanatomical Fidelity and the Receptor Question

The accumulated PFF mouse data establish four propositions about α-synuclein propagation. First, pathology spreads from inoculation site to anatomically connected sites on a timescale of weeks to months. Second, the spread respects synaptic connectivity rather than spatial proximity. Third, both anterograde and retrograde directions of spread are observed, with the dominant direction varying by region. Fourth, the spread is mechanistically distinct from passive diffusion or extracellular fluid flow: ablation of specific projections (e.g., vagotomy) blocks the corresponding propagation.

These propositions left a critical mechanistic question unresolved through approximately 2020: by what cellular machinery did extracellular α-synuclein gain access to the cytoplasm of recipient neurons? Multiple receptor candidates were proposed — heparan sulfate proteoglycans (HSPGs), LAG3, neurexin-1β, and members of the LDL receptor family. The 2022 Chen–Bu paper in *Molecular Neurodegeneration* identified LRP1 as a master neuronal receptor for α-synuclein uptake and propagation,[27] integrating the Lee laboratory's propagation phenomenology with a specific molecular receptor mechanism. Earlier candidate receptors — LAG3 (Mao 2016)[28] and HSPGs (Holmes 2013)[29] — likely contribute, with the field now

converging on a multi-receptor model in which different uptake machinery dominates in different cellular contexts.

The neuroanatomical fidelity of the PFF model has therapeutic implications the field is only beginning to operationalize. If pathology propagates along defined projections, then identifying the originating seed site is critical for therapeutic timing — a propagation blocker administered after the cortical projections have been seeded cannot reverse the seeded pathology, only halt further advance. The clinical implication is that biomarkers capable of detecting propagation in vivo — and ideally of identifying the originating site — would transform the therapeutic-trial design space.

Chapter 4: α-Synuclein Strains — MSA vs PD/DLB

The fourth foundational contribution of the Lee–Trojanowski program addresses a question implicit but unresolved in the early synucleinopathy framework: why do three disorders that share a single misfolded protein produce such different clinical syndromes, anatomical distributions of pathology, and cellular tropism? The 2018 Peng–Lee *Nature* paper supplied an answer that has come to define the modern understanding: α-synuclein exists not as a single pathological conformer but as distinct *strains*, with the PD/DLB strain and the MSA strain exhibiting different conformational signatures, different cellular targets, and different transmission kinetics.

4.1 The 2018 Peng Strain Discrimination

The Peng, Gathagan, Covell, Trojanowski, Lee *Nature* paper of 2018, *"Cellular milieu imparts distinct pathological α-synuclein strains in α-synucleinopathies,"*[8] reported that α-synuclein extracted from postmortem MSA brain (glial cytoplasmic inclusions) and from postmortem PD brain (Lewy bodies) exhibited fundamentally different biological and biochemical properties when used as seeds in cell-culture and mouse-brain inoculation experiments. MSA-derived α-synuclein produced rapid, aggressive synucleinopathy in mice with kinetics approximately twenty-fold faster than PD-derived material. MSA-derived seeds preferentially produced oligodendroglial pathology in some contexts; PD-derived seeds preferentially produced neuronal pathology. The conformational signatures of the resulting inclusions, assessed by conformation-sensitive antibodies and by proteolytic fingerprinting, were stably distinguishable.

Critically, the strain identity was self-propagating. Inoculation of MSA-derived material into a wild-type recipient mouse generated MSA-like pathology that could be subsequently re-extracted and used to seed a second recipient, producing the same MSA-like pathology. The strain identity was thus heritable across passages — the molecular hallmark of true prion

strains, established originally for PrP^Sc by Prusiner's collaborators in the 1990s. The Peng paper thus extended to α-synuclein the strain framework Prusiner had developed for the canonical prions, and Diamond had subsequently extended to tau.[30]

4.2 The 2020 Schweighauser Cryo-EM Structures

If the Peng paper established strain distinction at the level of biology and kinetics, the 2020 Schweighauser, Shi, Tarutani *Nature* paper from the Goedert and Scheres laboratories[9] established it at the level of atomic structure. The investigators applied cryo-electron microscopy to α-synuclein filaments extracted directly from postmortem MSA brain tissue and resolved the structures at near-atomic resolution (approximately 2.6 Å). The resulting structures revealed that MSA filaments exhibit a previously unknown fold — distinct from any of the recombinant α-synuclein structures reported up to that point, and distinct from preliminary structures of PD-derived filaments. The MSA filament was composed of two protofilaments arranged with a specific inter-protofilament interface; the protomer fold itself involved a specific arrangement of β-strands that had not been observed in any in-vitro-generated recombinant filament.

The structural finding was decisive in two respects. First, it confirmed that the Peng biological strain distinction reflected a fundamental difference in molecular architecture, not merely a difference in seeding kinetics or post-translational modification. Second, it demonstrated that fibrils extracted from human brain differ structurally from in-vitro-generated recombinant fibrils, raising the question of whether the standard recombinant-PFF model fully captures the conformational diversity of human disease. The Yang 2022 cryo-EM analysis of Lewy-body-derived α-synuclein from PD/DLB confirmed that the PD/DLB fold is structurally distinct from the MSA fold,[31] completing the molecular validation of the two-strain framework at atomic resolution.

4.3 Cellular Tropism and the Oligodendrocyte Question

The strain framework supplies a partial answer to one of the longest-standing puzzles in MSA biology: why oligodendrocytes accumulate α-synuclein pathology when they themselves express little to no α-synuclein under physiological conditions. The Peng work suggests that the MSA strain exhibits preferential affinity for the oligodendroglial cellular environment — either through preferred uptake by oligodendrocytes or through preferred templating of whatever α-synuclein is available within that compartment. The mechanism is incompletely understood but represents a tractable research target.

The strain framework also reframes MSA clinically. Prior to 2018, MSA was sometimes conceptualized as an aggressive or atypical variant of PD. The strain distinction implies that MSA is a categorically distinct disorder driven by a structurally different pathological

species. The therapeutic implication is that drugs developed against the PD/DLB α-synuclein strain may not act on the MSA strain, and vice versa. Strain-specific antibodies and small molecules may be required for the two disorders, complicating the clinical-trial landscape but also opening the prospect of strain-targeted precision therapeutics.

4.4 Strain Diversity Beyond the Two-Strain Framework

The two-strain framework — PD/DLB versus MSA — is almost certainly a simplification. Subsequent work has identified conformational sub-strains within both major categories, with potential variation across clinical subtypes (tremor-predominant versus postural-instability-predominant PD; cerebellar versus parkinsonian MSA), across genetic backgrounds (*SNCA*-mutation-driven versus sporadic), and across post-translational modification profiles. Whether these represent true distinct strains or variations within a continuous conformational landscape remains an active research question. The cryo-EM structural pipeline established by the Goedert–Scheres collaboration, applied systematically across postmortem cohorts, will likely resolve this question over the next several years.

> **What remains uncertain:** *Whether the strain landscape of α-synuclein admits a finite number of discrete pathological folds — analogous to the well-defined PrP^Sc strain set established by Prusiner's collaborators — or whether the landscape is continuous and disease-state-dependent, with each patient's α-synuclein pathology occupying a slightly different position. The therapeutic implication is substantial: a discrete strain framework supports strain-targeted antibodies; a continuous landscape supports broader-spectrum conformational-class antibodies.*

Chapter 5: Therapeutic Implications and Open Questions

The molecular framework the Lee–Trojanowski program has assembled — identification of the pathological protein, a reproducible model of its propagation, demonstration of its trans-synaptic spread, and resolution of its strain diversity — has driven the modern era of synucleinopathy therapeutics. Multiple therapeutic strategies have entered clinical evaluation; most have struggled; the strain framework and the spread paradigm together explain why, and point toward the next generation of approaches.

5.1 α-Synuclein Immunotherapy: Prasinezumab and Cinpanemab

The dominant therapeutic strategy of the past decade has been passive immunotherapy targeting extracellular α-synuclein. The two highest-profile programs — Roche's prasinezumab (PRX002, originally developed by Prothena) and Biogen's cinpanemab (BIIB054) — entered Phase II evaluation in early Parkinson's disease populations on the rationale that antibody-

mediated clearance of extracellular α-synuclein would interrupt trans-synaptic propagation and slow disease progression.

The PASADENA Phase II trial of prasinezumab in early PD failed to meet its primary endpoint of slowing motor progression on the MDS-UPDRS Part III at 52 weeks, though subgroup analyses suggested possible benefit in subpopulations with more rapidly progressive disease.[32] The SPARK Phase II trial of cinpanemab failed to demonstrate any clinical benefit and was discontinued.[33] Both failures prompted a reassessment of the immunotherapeutic strategy in light of what the Lee–Trojanowski framework actually predicts.

Three explanations are now widely discussed. First, the timing of administration may have been too late — by the time of clinical PD diagnosis, propagation has likely advanced extensively through the brainstem and into the cortex, and a propagation blocker administered at that stage can halt further spread but cannot reverse seeded pathology. Second, the antibodies may not engage the dominant pathological conformer; both prasinezumab and cinpanemab were developed against in-vitro-generated or oligomeric α-synuclein, and the brain-derived strain may differ structurally from the immunogen, as the cryo-EM data now suggest. Third, the antibodies' brain penetration was modest, with CSF concentrations likely insufficient to neutralize the propagating pool. The next generation of immunotherapeutics — strain-specific antibodies, brain-penetrant antibody formats, and combination approaches with delivery-enhancing technologies — addresses each of these explanations in turn.

5.2 Small-Molecule Aggregation Inhibitors

An alternative therapeutic strategy targets the aggregation step itself with small molecules. NPT200-11 (later NPT520-34) from UCB/Neuropore was advanced into early clinical evaluation as an oral small molecule that interacts directly with α-synuclein and modulates its assembly; the development pathway has been slow and the clinical data limited.[34] Anle138b, a diphenylpyrazole compound developed by Christian Griesinger's group at the Max Planck Institute, has demonstrated activity in PFF mouse models and entered Phase I clinical evaluation; Phase II trials in MSA and PD are under way.[35] The small-molecule strategy is theoretically attractive because oral administration and brain penetration are far easier to engineer than for antibodies, but the structural diversity of α-synuclein conformers raises the same strain-specificity question that the antibody programs confront.

5.3 SNCA-Lowering Antisense Oligonucleotides

If extracellular antibody therapy targets the propagating pool and small molecules target the aggregation step, a third strategy targets the substrate itself: lowering α-synuclein production by antisense oligonucleotide (ASO) suppression of *SNCA* expression. Biogen's BIIB101 (in collaboration with Ionis Pharmaceuticals) is in early clinical evaluation in MSA.[36] The

strategy depends on the assumption that reducing substrate availability will reduce templating efficiency — a hypothesis supported by both the Lee laboratory's PFF data (which require endogenous α-synuclein to template) and by the gene-dosage effect of *SNCA* multiplication in familial PD. The therapeutic risk is that α-synuclein has physiological functions in synaptic-vesicle dynamics whose disruption may produce on-target adverse effects.

5.4 The Intracerebral Propagation-Blockade Frontier

The most direct therapeutic implication of the trans-synaptic spread paradigm is the prospect of intracerebral propagation blockade — a molecule, administered directly into the CSF or via cell-permeable delivery, that selectively occupies the receptor by which extracellular α-synuclein gains entry into recipient neurons. The Chen–Bu identification of LRP1 as a master neuronal receptor for α-synuclein uptake[27] supplies a candidate target; LRP1 ligand-binding antagonists tailored to the α-synuclein interaction domain are now in preclinical development. The structural prerequisite is resolution of the α-synuclein–LRP1 binding interface at sufficient atomic detail to permit selective antagonist design without disturbing LRP1's APOE-binding and Aβ-clearance functions.

5.5 Open Questions for the Next Decade

Several questions stand unresolved at the close of the founding Lee–Trojanowski era. First, what is the earliest seeding site of human synucleinopathy? The olfactory bulb (Rey, Brundin), the enteric nervous system (Kim, Borghammer), and the locus coeruleus (consistent with Braak Stage 2) are competing candidates, with substantial overlap across cases. The clinical implications differ: a gut-first disease admits peripheral therapeutic intervention; a brain-first disease does not. Second, how do strains arise? The Peng work demonstrates that distinct strains exist and propagate stably; it does not explain why some patients develop the MSA strain and others the PD/DLB strain. Genetic background, cellular environment, and stochastic conformational selection during initial aggregation all likely contribute, but the relative weight of each is unknown. Third, can strain identity be inferred from CSF or plasma biomarkers in living patients? Recent advances in seed-amplification assays (RT-QuIC and PMCA adapted for α-synuclein) suggest that strain identity may be detectable from CSF α-synuclein with sufficient sensitivity, opening the prospect of biomarker-guided strain-specific therapy.[37] Fourth, what is the relationship between α-synuclein, lipid metabolism, and synaptic vesicle dynamics — the physiological functions whose disruption may precede or accompany pathological aggregation?

Conclusion

The research program of Virginia Lee and the late John Trojanowski at the University of Pennsylvania Center for Neurodegenerative Disease Research has, across nearly three decades, supplied the molecular biology of α-synuclein in disease. The 1997–1998 identification of α-synuclein as the Lewy body protein unified PD, DLB, and MSA under a single molecular framework. The 2011 Volpicelli-Daley cell-culture PFF model and the 2012 Luk in vivo translation supplied the tractable laboratory system that displaced an earlier generation of transgenic overexpression models and became the field's global standard. The systematic mapping of trans-synaptic spread along anatomical projections — extended by Brundin in the olfactory system and by the Dawson laboratory in the gut-to-brain axis — supplied the experimental architecture for the prion-like paradigm Prusiner had theorized and Walker–Jucker had demonstrated for the broader spectrum of proteinopathies. The 2018 Peng strain framework and the 2020 Schweighauser cryo-EM structures established that the synucleinopathies are not stages of a single disease but distinct strain-defined disorders with characteristic structural signatures.

Read together with the Diamond program in tauopathy — whose strain-discrimination, cell-line-based propagation assays, and seeding-competent species framework supplied the parallel molecular biology for tau — the Lee–Trojanowski program completes the synucleinopathy axis of the modern templated-misfolding paradigm.[30] Where Prusiner[10] supplied the conceptual unification and Walker–Jucker[11] supplied the experimental seeding architecture, Lee–Trojanowski supplied the protein-specific molecular biology for α-synuclein. The recent identification of LRP1 by the Bu laboratory[27] as the neuronal receptor mediating α-synuclein uptake supplies the receptor-level mechanism by which the trans-synaptic spread the Lee laboratory documented phenomenologically actually occurs. These five research programs — Prusiner, Walker–Jucker, Lee–Trojanowski, Diamond, and Bu — constitute a single integrated causal architecture for the templated-misfolding disorders.

The therapeutic implications follow directly. The PFF model is the field's workhorse for testing candidate disease-modifying drugs in vivo. The strain framework is the field's diagnostic future — patients may eventually be stratified by α-synuclein strain identity and receive strain-targeted therapy. The trans-synaptic spread paradigm establishes that the therapeutic window for propagation blockade is necessarily early, before the propagation cascade has saturated the cortical network. The failures of prasinezumab and cinpanemab in late-disease populations are precisely what the integrated framework predicts; the next generation of programs — strain-specific antibodies, receptor-blocking small molecules, and brain-penetrant delivery formats — engages the lessons the failures have taught.

John Trojanowski's death in February 2022 closed the founding partnership but not the program. Virginia Lee continues the laboratory's work at CNDR, and the conceptual edifice the

partnership constructed has become the operating framework of an entire subfield. The molecular biology of α-synuclein in disease is now sufficiently well-resolved that the unanswered questions are tractable rather than fundamental. Whether the field can translate that molecular resolution into clinical disease modification is the work of the next decade.

Works Cited

1. Spillantini, M.G., Schmidt, M.L., Lee, V.M.-Y., Trojanowski, J.Q., Jakes, R., & Goedert, M. (1997). α-Synuclein in Lewy bodies. *Nature*, 388(6645), 839–840. *doi.org/10.1038/42166*

2. Spillantini, M.G., Crowther, R.A., Jakes, R., Hasegawa, M., & Goedert, M. (1998). α-Synuclein in filamentous inclusions of Lewy bodies from Parkinson's disease and dementia with Lewy bodies. *Proceedings of the National Academy of Sciences USA*, 95(11), 6469–6473. *doi.org/10.1073/pnas.95.11.6469*

3. Polymeropoulos, M.H., Lavedan, C., Leroy, E., Ide, S.E., Dehejia, A., Dutra, A., et al. (1997). Mutation in the α-synuclein gene identified in families with Parkinson's disease. *Science*, 276(5321), 2045–2047. *doi.org/10.1126/science.276.5321.2045*

4. Volpicelli-Daley, L.A., Luk, K.C., Patel, T.P., Tanik, S.A., Riddle, D.M., Stieber, A., Meaney, D.F., Trojanowski, J.Q., & Lee, V.M.-Y. (2011). Exogenous α-synuclein fibrils induce Lewy body pathology leading to synaptic dysfunction and neuron death. *Neuron*, 72(1), 57–71. *doi.org/10.1016/j.neuron.2011.08.033*

5. Luk, K.C., Kehm, V., Carroll, J., Zhang, B., O'Brien, P., Trojanowski, J.Q., & Lee, V.M.-Y. (2012). Pathological α-synuclein transmission initiates Parkinson-like neurodegeneration in nontransgenic mice. *Science*, 338(6109), 949–953. *doi.org/10.1126/science.1227157*

6. Luk, K.C., Kehm, V.M., Zhang, B., O'Brien, P., Trojanowski, J.Q., & Lee, V.M.-Y. (2012). Intracerebral inoculation of pathological α-synuclein initiates a rapidly progressive neurodegenerative α-synucleinopathy in mice. *Journal of Experimental Medicine*, 209(5), 975–986. *doi.org/10.1084/jem.20112457*

7. Braak, H., Del Tredici, K., Rüb, U., de Vos, R.A.I., Jansen Steur, E.N.H., & Braak, E. (2003). Staging of brain pathology related to sporadic Parkinson's disease. *Neurobiology of Aging*, 24(2), 197–211. *doi.org/10.1016/S0197-4580(02)00065-9*

8. Peng, C., Gathagan, R.J., Covell, D.J., Medellin, C., Stieber, A., Robinson, J.L., Zhang, B., Pitkin, R.M., Olufemi, M.F., Luk, K.C., Trojanowski, J.Q., & Lee, V.M.-Y. (2018). Cellular milieu imparts distinct pathological α-synuclein strains in α-synucleinopathies. *Nature*, 557(7706), 558–563. *doi.org/10.1038/s41586-018-0104-4*

9. Schweighauser, M., Shi, Y., Tarutani, A., Kametani, F., Murzin, A.G., Ghetti, B., Hasegawa, M., Goedert, M., & Scheres, S.H.W. (2020). Structures of α-synuclein filaments from multiple system atrophy. *Nature*, 585(7825), 464–469. *doi.org/10.1038/s41586-020-2317-6*

10. Prusiner, S.B. (2012). A unifying role for prions in neurodegenerative diseases. *Science*, 336(6088), 1511–1513. *doi.org/10.1126/science.1222951*

11. Jucker, M., & Walker, L.C. (2013). Self-propagation of pathogenic protein aggregates in neurodegenerative diseases. *Nature*, 501(7465), 45–51. *doi.org/10.1038/nature12481*

12. Maroteaux, L., Campanelli, J.T., & Scheller, R.H. (1988). Synuclein: a neuron-specific protein localized to the nucleus and presynaptic nerve terminal. *Journal of Neuroscience*, 8(8), 2804–2815. *doi.org/10.1523/JNEUROSCI.08-08-02804.1988*

13. Wakabayashi, K., Yoshimoto, M., Tsuji, S., & Takahashi, H. (1998). α-Synuclein immunoreactivity in glial cytoplasmic inclusions in multiple system atrophy. *Neuroscience Letters*, 249(2–3), 180–182. *doi.org/10.1016/S0304-3940(98)00407-8*

14. Kordower, J.H., Chu, Y., Hauser, R.A., Freeman, T.B., & Olanow, C.W. (2008). Lewy body-like pathology in long-term embryonic nigral transplants in Parkinson's disease. *Nature Medicine*, 14(5), 504–506. *doi.org/10.1038/nm1747*

15. Li, J.-Y., Englund, E., Holton, J.L., Soulet, D., Hagell, P., Lees, A.J., Lashley, T., Quinn, N.P., Rehncrona, S., Björklund, A., Widner, H., Revesz, T., Lindvall, O., & Brundin, P. (2008). Lewy bodies in grafted neurons in subjects with Parkinson's disease suggest host-to-graft disease propagation. *Nature Medicine*, 14(5), 501–503. *doi.org/10.1038/nm1746*

16. Desplats, P., Lee, H.-J., Bae, E.-J., Patrick, C., Rockenstein, E., Crews, L., Spencer, B., Masliah, E., & Lee, S.-J. (2009). Inclusion formation and neuronal cell death through neuron-to-neuron transmission of α-synuclein. *Proceedings of the National Academy of Sciences USA*, 106(31), 13010–13015. *doi.org/10.1073/pnas.0903691106*

17. Krüger, R., Kuhn, W., Müller, T., Woitalla, D., Graeber, M., Kösel, S., Przuntek, H., Epplen, J.T., Schöls, L., & Riess, O. (1998). Ala30Pro mutation in the gene encoding α-synuclein in Parkinson's disease. *Nature Genetics*, 18(2), 106–108. *doi.org/10.1038/ng0298-106*

18. Singleton, A.B., Farrer, M., Johnson, J., Singleton, A., Hague, S., Kachergus, J., et al. (2003). α-Synuclein locus triplication causes Parkinson's disease. *Science*, 302(5646), 841. *doi.org/10.1126/science.1090278*

19. Kim, S., Kwon, S.-H., Kam, T.-I., Panicker, N., Karuppagounder, S.S., Lee, S., Lee, J.H., Kim, W.R., Kook, M., Foss, C.A., Shen, C., Lee, H., Kulkarni, S., Pasricha, P.J., Lee, G., Pomper, M.G., Dawson, V.L., Dawson, T.M., & Ko, H.S. (2019). Transneuronal propagation of pathologic α-synuclein from the gut to the brain models Parkinson's disease. *Neuron*, 103(4), 627–641.e7. *doi.org/10.1016/j.neuron.2019.05.035*

20. Borghammer, P., & Van Den Berge, N. (2019). Brain-first versus gut-first Parkinson's disease: a hypothesis. *Journal of Parkinson's Disease*, 9(s2), S281–S295. *doi.org/10.3233/JPD-191721*

21. Volpicelli-Daley, L.A., Luk, K.C., & Lee, V.M.-Y. (2014). Addition of exogenous α-synuclein preformed fibrils to primary neuronal cultures to seed recruitment of endogenous α-synuclein to Lewy body and Lewy neurite-like aggregates. *Nature Protocols*, 9(9), 2135–2146. *doi.org/10.1038/nprot.2014.143*

22. Fujiwara, H., Hasegawa, M., Dohmae, N., Kawashima, A., Masliah, E., Goldberg, M.S., Shen, J., Takio, K., & Iwatsubo, T. (2002). α-Synuclein is phosphorylated in synucleinopathy lesions. *Nature Cell Biology*, 4(2), 160–164. *doi.org/10.1038/ncb748*

23. Tarutani, A., Suzuki, G., Shimozawa, A., Nonaka, T., Akiyama, H., Hisanaga, S., & Hasegawa, M. (2016). The effect of fragmented pathogenic α-synuclein seeds on prion-like propagation. *Journal of Biological Chemistry*, 291(36), 18675–18688. *doi.org/10.1074/jbc.M116.734707*

24. Luk, K.C., Covell, D.J., Kehm, V.M., Zhang, B., Song, I.Y., Byrne, M.D., Pitkin, R.M., Decker, S.C., Trojanowski, J.Q., & Lee, V.M.-Y. (2016). Molecular and biological compatibility with host α-synuclein influences fibril pathogenicity. *Cell Reports*, 16(12), 3373–3387. *doi.org/10.1016/j.celrep.2016.08.053*

25. Henderson, M.X., Cornblath, E.J., Darwich, A., Zhang, B., Brown, H., Gathagan, R.J., Sandler, R.M., Bassett, D.S., Trojanowski, J.Q., & Lee, V.M.-Y. (2019). Spread of α-synuclein pathology through the brain connectome is modulated by selective vulnerability and predicted by network analysis. *Nature Neuroscience*, 22(8), 1248–1257. *doi.org/10.1038/s41593-019-0457-5*

26. Rey, N.L., Petit, G.H., Bousset, L., Melki, R., & Brundin, P. (2013). Transfer of human α-synuclein from the olfactory bulb to interconnected brain regions in mice. *Acta Neuropathologica*, 126(4), 555–573. *doi.org/10.1007/s00401-013-1160-3*

27. Chen, K., Martens, Y.A., Meneses, A., Ryu, D.H., Lu, W., Raulin, A.C., Li, F., Zhao, J., Chen, Y., Jin, Y., Linares, C., Goeminne, L.J.E., Wang, N., Liu, C.-C., Bu, G., & Painter, M.M. (2022). LRP1 is a neuronal receptor for α-synuclein uptake and spread. *Molecular Neurodegeneration*, 17, 57. *doi.org/10.1186/s13024-022-00560-w*

28. Mao, X., Ou, M.T., Karuppagounder, S.S., Kam, T.-I., Yin, X., Xiong, Y., Ge, P., Umanah, G.E., Brahmachari, S., Shin, J.-H., Kang, H.C., Zhang, J., Xu, J., Chen, R., Park, H., Andrabi, S.A., Kang, S.U., Gonçalves, R.A., Liang, Y., Zhang, S., Qi, C., Lam, S., Keiler, J.A., Tyson, J., Kim, D., Panicker, N., Yun, S.P., Workman, C.J., Vignali, D.A.A., Dawson, V.L., Ko, H.S., & Dawson, T.M. (2016). Pathological α-synuclein transmission initiated by binding lymphocyte-activation gene 3. *Science*, 353(6307), aah3374. *doi.org/10.1126/science.aah3374*

29. Holmes, B.B., DeVos, S.L., Kfoury, N., Li, M., Jacks, R., Yanamandra, K., Ouidja, M.O., Brodsky, F.M., Marasa, J., Bagchi, D.P., Kotzbauer, P.T., Miller, T.M., Papy-Garcia, D., & Diamond, M.I. (2013). Heparan sulfate proteoglycans mediate internalization and propagation of specific proteopathic seeds. *Proceedings of the National Academy of Sciences USA*, 110(33), E3138–E3147. *doi.org/10.1073/pnas.1301440110*

30. Sanders, D.W., Kaufman, S.K., DeVos, S.L., Sharma, A.M., Mirbaha, H., Li, A., Barker, S.J., Foley, A.C., Thorpe, J.R., Serpell, L.C., Miller, T.M., Grinberg, L.T., Seeley, W.W., & Diamond, M.I. (2014). Distinct tau prion strains propagate in cells and mice and define different tauopathies. *Neuron*, 82(6), 1271–1288. *doi.org/10.1016/j.neuron.2014.04.047*

31. Yang, Y., Shi, Y., Schweighauser, M., Zhang, X., Kotecha, A., Murzin, A.G., Garringer, H.J., Cullinane, P.W., Saito, Y., Foroud, T., Warner, T.T., Hasegawa, K., Vidal, R., Murayama, S., Revesz, T., Ghetti, B., Hasegawa, M., Lashley, T., Scheres, S.H.W., & Goedert, M. (2022). Structures of α-synuclein filaments from human brains with Lewy pathology. *Nature*, 610(7933), 791–795. *doi.org/10.1038/s41586-022-05319-3*

32. Pagano, G., Taylor, K.I., Anzures-Cabrera, J., Marchesi, M., Simuni, T., Marek, K., Postuma, R.B., Pavese, N., Stocchi, F., Azulay, J.-P., Mollenhauer, B., López-Manzanares, L., Russell, D.S., Boyd, J.T., Nicholas, A.P., Luquin, M.R., Hauser, R.A., Gasser, T., Poewe, W., et al. (2022). Trial of prasinezumab in early-stage Parkinson's disease. *New England Journal of Medicine*, 387(5), 421–432. *doi.org/10.1056/NEJMoa2202867*

33. Lang, A.E., Siderowf, A.D., Macklin, E.A., Poewe, W., Brooks, D.J., Fernandez, H.H., Rascol, O., Giladi, N., Stocchi, F., Tanner, C.M., Postuma, R.B., Simon, D.K., Tolosa, E., Mollenhauer, B., Cedarbaum, J.M., Fraser, K., Xiao, J., Evans, K.C., Graham, D.L., Sapir, I., et al. (2022). Trial of cinpanemab in early Parkinson's disease. *New England Journal of Medicine*, 387(5), 408–420. *doi.org/10.1056/NEJMoa2203395*

34. Price, D.L., Khan, A., Angers, R., Cardenas, A., Prato, M.K., Bani, M., Bonhaus, D.W., Citron, M., & Biere, A.L. (2018). In vivo effects of the α-synuclein misfolding inhibitor minzasolmin supports clinical development in Parkinson's disease. *npj Parkinson's Disease*, 4, 13. *doi.org/10.1038/s41531-018-0044-6*

35. Wagner, J., Ryazanov, S., Leonov, A., Levin, J., Shi, S., Schmidt, F., Prix, C., Pan-Montojo, F., Bertsch, U., Mitteregger-Kretzschmar, G., Geissen, M., Eiden, M., Leidel, F., Hirschberger, T., Deeg, A.A., Krauth, J.J., Zinth, W., Tavan, P., Pilger, J., Zweckstetter, M., et al. (2013). Anle138b: a novel oligomer modulator for disease-modifying therapy of neurodegenerative diseases such as prion and Parkinson's disease. *Acta Neuropathologica*, 125(6), 795–813. *doi.org/10.1007/s00401-013-1114-9*

36. Cole, T.A., Zhao, H., Collier, T.J., Sandoval, I., Sortwell, C.E., Steece-Collier, K., Daley, B.F., Booms, A., Lipton, J., Welch, M., Berman, M., Jandreski, L., Graham, D., Weihofen, A., Celano, S., Schulz, E., Cole-Strauss, A., Luna, E., Quach, D., Mohan, A., et al. (2021). α-Synuclein antisense oligonucleotides as a disease-modifying therapy for Parkinson's disease. *JCI Insight*, 6(5), e135633. *doi.org/10.1172/jci.insight.135633*

37. Shahnawaz, M., Mukherjee, A., Pritzkow, S., Mendez, N., Rabadia, P., Liu, X., Hu, B., Schmeichel, A., Singer, W., Wu, G., Tsai, A.-L., Shirani, H., Nilsson, K.P.R., Low, P.A., & Soto, C. (2020). Discriminating α-synuclein strains in Parkinson's disease and multiple system atrophy. *Nature*, 578(7794), 273–277. *doi.org/10.1038/s41586-020-1984-7*

38. Trojanowski, J.Q., & Lee, V.M.-Y. (2003). Parkinson's disease and related α-synucleinopathies are brain amyloidoses. *Annals of the New York Academy of Sciences*, 991(1), 107–110. *doi.org/10.1111/j.1749-6632.2003.tb07468.x*

39. Giasson, B.I., Duda, J.E., Quinn, S.M., Zhang, B., Trojanowski, J.Q., & Lee, V.M.-Y. (2002). Neuronal α-synucleinopathy with severe movement disorder in mice expressing A53T human α-synuclein. *Neuron*, 34(4), 521–533. *doi.org/10.1016/S0896-6273(02)00682-7*

40. Lee, V.M.-Y., Giasson, B.I., & Trojanowski, J.Q. (2004). More than just two peas in a pod: common amyloidogenic properties of tau and α-synuclein in neurodegenerative diseases. *Trends in Neurosciences*, 27(3), 129–134. *doi.org/10.1016/j.tins.2004.01.007*

41. Brundin, P., Melki, R., & Kopito, R. (2010). Prion-like transmission of protein aggregates in neurodegenerative diseases. *Nature Reviews Molecular Cell Biology*, 11(4), 301–307. *doi.org/10.1038/nrm2873*

42. Prusiner, S.B., Woerman, A.L., Mordes, D.A., Watts, J.C., Rampersaud, R., Berry, D.B., Patel, S., Oehler, A., Lowe, J.K., Kravitz, S.N., Geschwind, D.H., Glidden, D.V., Halliday, G.M., Middleton, L.T., Gentleman, S.M., Grinberg, L.T., & Giles, K. (2015). Evidence for α-synuclein prions causing multiple system atrophy in humans with parkinsonism. *Proceedings of the National Academy of Sciences USA*, 112(38), E5308–E5317. *doi.org/10.1073/pnas.1514475112*