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Targeting α-synuclein for treating Parkinson's disease: mechanistic and therapeutic considerations

Benjamin Dehay[#1], **Mathieu Bourdenx**[#1], **Philippe Gorry**[2], **Serge Przedborski**[3], **Miquel Vila**[4,5,6], **Stephane Hunot**[7,8,9,10], **Andrew Singleton**[11], **C. Warren Olanow**[12], **Kalpana M. Merchant**[13], **Erwan Bezard**[#,1], **Gregory A. Petsko**[14], and **Wassilios G. Meissner**[1]

[1]Institute of Neurodegenerative Diseases, University of Bordeaux, Centre National de la Recherche Scientifique Unité Mixte de Recherche 5293, 33076 Bordeaux, France

[2]Research Unit of Theoretical & Applied Economics, University of Bordeaux, Centre National de la Recherche Scientifique Unité Mixte de Recherche 5113, 33608 Pessac, France

[3]Departments of Neurology, Pathology and Cell Biology, and the Center for Motor Neuron Biology and Disease, Columbia University, New York, NY 10032, USA

[4]Neurodegenerative Diseases Research Group, Vall d'Hebron Research Institute, Centro Investigación Biomédica en Red Enfermedades Neurodegenerativas, 08035 Barcelona, Spain

[5]Department of Biochemistry and Molecular Biology, Autonomous University of Barcelona, 08193 Bellaterra, Barcelona, Spain

[6]Catalan Institution for Research and Advanced Studies, 08010 Barcelona, Spain

[7]ICM, Paris, France; Sorbonne Universités

[#]To whom correspondence should be addressed: Erwan Bezard, Institut des Maladies Neurodégénératives, Université de Bordeaux, UMR 5293, Bât 3B 1er étage, 146 rue Léo Saignat, 33076 Bordeaux cedex; phone: +33 5 57 57 15 40; erwan.bezard@u-bordeaux.fr. .

Contributions

EB, WGM, BD and MB were responsible for the conception of the article. BD, MB and PG did the literature review. PG created the tables. BD and MB created the figures. BD, MB, EB, WGM wrote the first draft article. BD, MB, PG, SP, MV, SH, AS, CWO, KMM, EB, GAP and WGM critically revised the entire manuscript and approved the final version.

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[8]UPMC Université Paris 06, UM 75, ICM, Paris, France

[9]CNRS, UMR 7225, ICM, Paris, France

[10]Inserm, U 1127, ICM, Paris, France

[11]Molecular Genetics Section and Laboratory of Neurogenetics, NIA, NIH, Bethesda, MD20892, USA

[12]Departments of Neurology and Neuroscience, Mount Sinai School of Medicine, New York, NY 10032, USA

[13]TransThera Consulting Co., Zionsville, IN, 46077, USA

[14]Department of Neurology and Feil Family Brain and Mind research Institute, Weill Cornell Medical College, New York NY 10021, USA

[#] These authors contributed equally to this work.

Summary

Progressive neuronal cell loss in a small subset of brainstem and mesencephalic nuclei and widespread aggregation of the α-synuclein protein in the form of Lewy bodies and Lewy neurites are neuropathological hallmarks of Parkinson's disease. Most cases occur sporadically, but mutations in several genes, including α-synuclein, are associated with disease development. The mechanisms driving neurodegeneration remain unknown, hence limiting therapeutic strategies aimed at blocking neuronal death. This review describes current evidence for a predominant role of α-synuclein in the pathogenesis of PD, as well as some of the most promising α-synuclein-based strategies currently in development for this incurable neurodegenerative disorder.

Keywords

Parkinson's disease; α-synuclein; therapeutic strategies; pipeline; clinical trials

Introduction

Over the past two decades, a myriad of studies have suggested a significant pathogenic role of α-synuclein (α-syn) in both familial and sporadic forms of Parkinson's disease (PD). PD belongs to the synucleinopathies, which includes dementia with Lewy bodies (DLB) and multiple system atrophy (MSA). PD is the second most common neurodegenerative disorder affecting 1 to 3% of the population over the age of 50 [1] and over 5 million people worldwide [2]. Classical motor signs of PD include bradykinesia, rigidity, resting tremor and gait disturbance with postural instability [3], primarily attributed to the dramatic loss of dopamine (DA)-containing neurons in the substantia nigra pars compacta (SNpc). In addition to DA neuronal cell loss, another pathological hallmark of PD is the presence of intraneuronal proteinaceous cytoplasmic inclusions, named Lewy bodies (LB), and dystrophic Lewy neurites (LN) that also contain α-syn deposits. The mechanisms leading to the formation and the pathogenic significance of these inclusions remain unknown.

To date, there is no existing neuroprotective or neurorestorative therapy for treating this chronic disorder. However, two key discoveries that occurred 17 years ago have dramatically impacted PD research: i) the identification of mutations in the gene encoding for α-syn (*SNCA*) in families with PD [4, 5] and ii) the demonstration that α-syn is a major component of Lewy pathology [4, 5]. Despite our increasing understanding of PD pathogenesis, the exact mechanisms of the progressive DA cell loss in the SNpc remain to be unraveled. Here, we review recent advances in understanding the pivotal role of α-syn in PD and stress the need to focus therapeutic development on this target.

Implication of α-syn in the disease process

α-Synuclein and Parkinson's disease—In 1912, F.J.H. Lewy first described the intraneuronal proteinaceous cytoplasmic inclusions which became the histopathological hallmark of PD [6]. Several years after this discovery, K.N. Tretiakoff named them the "Lewy Body". In the early 90's, α-syn was identified as the precursor of the non-amyloid component (NAC) of Alzheimer's disease (AD) amyloid plaques [7-9]. The first link between α-syn and PD appeared in 1997 with the identification of point mutations in the *SNCA* gene in familial forms of PD (*PARK1* locus) [4]. Polymeropoulos and colleagues identified the *SNCA* gene coding for α-syn on chromosome 4q21-q23 and described A53T as the first point mutation causing autosomal-dominant PD [4]. Six missense mutations in *SNCA* are now associated with autosomal dominant PD: p.A53T, p.A30P, p.E46K, p.H50Q, p.G51D, p.A53E [4, 10-15] (Figure 1). These mutations are extremely rare with only a few families identified for each mutation. While the p.A30P mutation induces a clinical picture close to sporadic disease, p.A53T, p.E46K, p.H50Q and the newly identified p.G51D and p.A53E mutations are characterized by an earlier onset of parkinsonism with rapid disease progression and additional clinical features such as hallucinations, dementia, pyramidal tract impairment and autonomic failure [4, 10-12, 15]. Neuropathological reports on autopsies of PD patients with p.A53T, p.A30P, p.E46K and p.G51D mutations described dopaminergic cell loss with extensive synucleinopathy in several brain regions [10, 12, 16, 17]. The subsequent identification of families with duplication or triplication of the *SNCA* gene (*PARK4* locus) strengthened the link between α-syn and PD, and indicated that increased levels of even the wild-type protein alone can cause the disease [18, 19]. The clinical phenotype of patients with *SNCA* triplication (i.e. early onset parkinsonism with dementia) is more severe than in those with *SNCA* duplication (i.e. close to idiopathic PD) suggesting a dose-dependent relationship between disease severity and *SNCA* gene dosage. The common genetic variability at the SNCA locus is a robust risk factor for disease.

α-Synuclein structure and function—Although the physiological function of α-syn remains to be fully elucidated, α-syn is implicated in modulating synaptic activity through regulation of synaptic-vesicle release (recently reviewed in [20]). α-Syn, a 14kDa protein (other isoforms however exist), is a member of a small family of three proteins: α-, β- (β-syn) and γ-synuclein (γ-syn) [2]. Three different domains can be defined in the 14kDa isoform of α-syn: i) an N-terminal domain (residues 1-60), ii) a central NAC domain (residues 61-95), and iii) a C-terminal domain (residues 96-140) (Figure 1). The N-terminal domain is characterized by repetitions of the four lysine-rich highly conserved motif "KTK(E/Q)GV", similar to lipid-binding motifs in amphipathic helical domains of

lipoproteins [21]. All known clinical mutations are present in this region, emphasizing the importance of this domain in the aggregation of α-syn. The NAC domain has a high content in hydrophobic amino acids, responsible for the aggregation-prone properties, while the C-terminal end is characterized by a high content in proline, aspartate and glutamate residues. α- and β-syn have a high sequence identity (around 90%) in the amino-terminal domain, while the NAC region of α-syn specifically contains a 12 amino acid motif (residues 71-82: "VTGVTAVAQKTV") that is a key element in the α-syn aggregation process, in particular its fibrillation (accumulation of β-sheet-rich aggregates) [22].

The native state of α-syn is extensively debated. While some reported that α-syn purified from human cells is a helically folded tetramer, others found that α-syn predominantly exist as an unfolded monomer [8, 23-26]. Taken together, these studies suggest that α-syn exists under various conformational shapes and oligomeric states in a dynamic equilibrium, modulated by factors either accelerating or inhibiting fibrillation (Figure 2). Disease-related mutations impact the aggregation dynamics [27-29]. The identification and characterization of the toxic α-syn species remain incomplete. Two hypotheses have been proposed: toxic species could be (i) amyloid-like insoluble fibrils, notably found in LB, or (ii) soluble, prefibrillar intermediates, such as oligomers or protofibrils (Figure 2). Several groups have sifted through the different states of α-syn aggregation and thoroughly examined the functional consequences of aggregate-associated toxicity. Winner and colleagues generated mutants unable to form fibrils while keeping an oligomeric state, and showed enhanced toxicity *in vivo* [30]. Increasing evidence from both *in vitro* and *in vivo* studies has corroborated that oligomeric species are the most relevant [26, 29-32] and suggests that LB may be protective and represent a form of aggresome [33]. While the different oligomeric types exist in a dynamic equilibrium and slowly convert into fibrils, Lashuel and coworkers recently proposed that annular oligomers are not on the pathway leading to amyloid formation and are therefore potentially toxic [2]. This result, if confirmed, suggests that stabilization of the amyloid pathway might be of therapeutic interest.

α-syn oligomeric species bind to lipids and increase mitochondrial, lysosomal and vesicular membrane permeability, a common feature of aggregation-prone proteins [32, 34-37]. Increasing membrane permeability leads to calcium influx, ion homeostasis disruption and cell death through caspase-3 activation [32]. Oligomers, but not monomers, can inhibit synaptic vesicle docking [38].

Several post-translational modifications (PTMs) of α-syn were characterized and their presence noted in LB pathology (for in-depth review see [21]). First, phosphorylation is the best-studied PTMs. Several studies characterized two sites of phosphorylation in α-syn, Ser87 and especially Ser129, which is phosphorylated in brains from PD patients [39, 40] as well as in several *in vivo* and *in vitro* models of PD [41]. It remains unclear whether phosphorylation of α-syn impacts the fibrillation process [42]. Among the different PTMs identified, Ser129 phosphorylated (pS129) α-syn is thought to be the dominant form of α-syn in LB [41]. Consistent with these findings, a recent unbiased top down proteomics study quantified various α-syn forms in the frontal cortex from PD cases and controls and demonstrated the presence of pS129 α-syn only in SDS-insoluble (LB-enriched) fraction of brain tissue [43]. However, the total amount of pS129 α-syn was quite variable between cases

and represented a minor fraction of unphosphorylated α-syn, raising the question around the validity of pS129 α-syn as a therapeutic target. Second, nitration of α-syn at residues Y39, Y125 and Y133 has been characterized in brains from patients with synucleinopathies [44]. Third, oxidation of α-syn could occur by way of oxidized derivatives of DA leading to a decrease in fibril formation and a subsequent increase in protofibril accumulation [45]. Both nitration and oxidation decrease the propensity of α-syn to form fibrils and stabilize oligomers, and might thus enhance toxicity [46, 47]. Truncated α-syn species have also been found in LB [41, 43]. Truncation typically occurs in the disordered C-terminal third of the protein, most frequently at or near position 121. Truncation is associated with an increased propensity of α-syn to form fibrils *in vitro* and with increased toxicity in overexpressing flies and rats [48, 49]. However, no compelling data correlate truncated α-syn levels and clinicopathology [43]. Additionally, we still lack direct *in vivo* evidence that inhibition of α-syn-cleaving proteases decreases cell death. Indeed, the physiological or pathological significance of α-syn cleavage remains equivocal. While most studies focus on the N-terminal peptide (the longest one) produced by cleavage, future studies should also consider the C-terminal peptide.

α-Synuclein degradation—Increased levels of aggregated α-syn in PD suggest that defective protein handling and clearance contribute to its pathogenesis. It is now established that α-syn is degraded both by the ubiquitin-proteasome system (UPS) and by the autophagy/lysosomal pathway (ALP) [50-52]. α-syn contains a chaperone-mediated autophagy (CMA) recognition motif ^{95}VKKDQ^{99} (KFERQ like) allowing interaction with cytosolic-hsc70 and translocation into the lysosome through the lysosome-associated membrane receptor protein, LAMP2a [52]. Interestingly, mutant (p.A30P and p.A53T) and DA-modified α-syn, unlike wild-type α-syn, fail to be released within the lysosomal lumen after binding to LAMP2a, hence clogging this autophagy translocation machinery and leading to the accumulation of CMA-clients, at least *in vitro* [52, 53]. Additionally, α-syn by itself may compromise macroautophagy [54, 55]. *In vivo* evidence suggests that normal soluble α-syn is primarily degraded by the UPS while more complex conformations, including aggregates are disposed of by the ALP [56]. Consistent with these observations, pathogenic depletion in proteasome components and lysosomes (i.e. loss of catalytic activity and decrease in lysosome number) has been observed in sporadic PD brains and in both toxic and genetic rodent models supporting the idea that defects in protein quality-control mechanism contribute to PD pathogenesis [57, 58]. A vicious cycle thus occurs in which protein accumulation impairs clearance of the protein thus promoting further accumulation ultimately leading to neurodegeneration.

The properties of mutant and post-translationally modified α-syn species suggest that α-syn-mediated toxicity could occur through several distinct pathways (Figure 3). Apart from its localization at presynaptic terminals, α-syn accumulation has also been associated with endoplasmic reticulum (ER) stress [59] and several organelle defects, e.g. mitochondrion or lysosome. Localization at the mitochondria-associated ER membrane has been recently reported [60]. Both WT and mutant forms of α-syn may impact mitochondrial morphology [60]. The aforementioned cellular defects could also be related to ageing, that remains the most prominent risk factor for PD [61]. Ageing itself leads to mitochondrial and lysosomal

impairments, altered calcium metabolism and production of reactive oxygen species [61]. Combination of ageing and PD alterations could lead to a cellular stress condition that interferes with intracellular clearance pathways, favor α-syn aggregation and contribute to the extracellular release of α-syn.

α-Synuclein spreading—In 2008, three independent studies reported that embryonic mesencephalic neurons grafted into the striatum of three PD patients, out of 8 investigated, develop LB-like structures (α-syn, ubiquitin and Thioflavin-S positive) in grafted neurons, 11, 14 and 16 years after brain surgery [62-64]. This observation suggested a "host-to-the-graft" transmission of LB pathology in the human brain and led to the speculation that cell-to-cell transmission of abnormal α-syn species contribute to PD pathogenesis. Relevant to this concept is the fact that PD patients exhibit α-syn-positive deposits in different brain regions, which, in some instances, are interconnected [65]. A variety of evidence now suggests that α-syn pathology initiates in the periphery (possibly gastro-intestinal system) with subsequent spread to the central nervous system. However, whether such an anatomical pattern reflects some sort of caudo-rostral transmission of abnormal α-syn species or merely a caudo-rostral gradient of neuronal sensitivity thresholds for developing α-syn-positive deposits remains to be established.

The exact mechanism underlying the initiation of α-syn misfolding and aggregation in a recipient cell remains unknown. *In vivo* studies have added a further piece to the puzzle with the observation that intracerebral inoculation of α-syn-derived from purified LB taken from the SNpc of PD patients [66] or *in vitro* recombinant pffs of α-syn [67] can promote LB-like pathology in host neurons of recipient animals. Other reviews on α-syn spreading are available that cover more examples that we can here [68], but we will pinpoint the unsolved questions that should be answered.

Evidence piles up to strengthen the concept that α-syn may self-propagate, thereby contributing to the progression and extension of PD. In this context, the existence of toxic vs. non-toxic α-syn strains could underlie the differences in disease propagation between individuals, cell types or synucleinopathies [69]. However, it has not been yet demonstrated whether the pathological conversion of endogenous α-syn triggered by PD-derived material or recombinant α-syn pffs may actually occur directly through a seeding prion process or rather indirectly as a general response to cellular stress. In addition, besides any potential pathogenic effect of intraneuronal α-syn, extracellular pathological α-syn is able to activate a deleterious microglial response that may also contribute to overall cell death and extension of the PD pathological process [70]. It also remains to determine what initiates the misfolding of α-syn and a prion-like cascade. Potential causes include genetic mutations and duplication/triplication (in rare instances), impaired clearance, or exposure to toxins or infectious agents. The latter has attracted much interest, as olfactory filaments are the only nerves directly exposed to the exterior environment. Terminals of the dorsal motor nucleus of the vagus nerve reside in the gastric mucosa just microns from the lumen. As these structures appear to be the first areas of the brain involved in the PD process in at least some patients, it is easy to imagine that they could readily be exposed to toxic or infectious agents. Finally, it is possible that misfolding of α-syn occurs randomly, and the initiation of a prion cascade is a function of multiple factors that might contribute differentially in different

individuals. Among several mechanisms, α-syn oligomers are thought to be the toxic species and the cause of the neurodegenerative process. Further, these oligomers would spread throughout the brain and induce α-syn pathology in interconnected structures. The toxicity of these pathogenic forms of α-syn, which remains to be unequivocally demonstrated, could be exerted through different intertwined mechanisms involving both cell autonomous (e.g. ion homeostasis perturbation, disruption of the mitochondrial network and/or impaired proteostasis) (Figure 3) and non-cell autonomous (e.g. neuro-inflammation) pathways. In line with the latter, aggregated and oxidatively modified α-syn holds interesting immunological features possibly involved in lethal neuro-immune interactions. In innate immunity, the Toll-like Receptor 2 has been identified as a major microglial receptor for neuron-released oligomeric α-syn and the ensuing inflammatory reaction [70]. Among the mechanisms involved in the neurotoxic microglial-associated innate immune response, the oxidative burst generated by these brain macrophages is central and most likely implicated in the post-translational modification of α-syn through nitration of Y125 and Y133 residues [71]. Interestingly, nitrated α-syn, unlike the native protein, can escape immune tolerance and generate a deleterious T helper cell response directed toward dopaminergic neurons as shown by immunization and passive transfer experiments in mice [72]. Although infiltrated T cell have been identified in the brain of PD patients, it remains to be proven that this adaptive immune response is related to pathological α-syn antigens [73]. Overall, it is tempting to speculate that any approaches targeting α-syn aggregation or propagation would indirectly impact on these immune responses but other, more direct, immunotherapeutics may also be considered.

Where do we come from and where are we going?

α-Syn-related publications have consistently grown since its relationship to PD was discovered in 1997 to reach 446 articles published in 2013 (Figure 4A). Interestingly, 50% of the articles were published in the last five years (Figure 4A). The increase in scientific publications is associated with an increasing α-syn-related patent deposit rate (Figure 4A). Since 1994, a total of 176 patent families have been filed, with an average of 20 patents per year in the last 3 years (Figure 4A). The majority of early patents were for diagnosis purposes and/or drug screening against α-syn. More recently, α-syn-related patents refer to anti-aggregation compounds, gene-silencing approaches and clearance strategies (Table 1), holding still a basic science status (Table 1). Conversely, a few have entered the pipeline but have not yet moved to clinical trials, suggesting that α-syn will be one of the main foci for biomarkers identification and disease-modifying strategies in the near future (Table 2). So far, 13 active α-syn-focused clinical trials are ongoing funded mostly by academic or non-industrial partners (Figure 4C **and** Table 3). Beside the increasing number of scientific publications and patents dealing with α-syn, the increasing number of economic press releases in large media underlines the rising interest for therapeutic strategies targeting α-syn (Figure 4B).

Strategies to combat α-synuclein toxicity

α-syn aggregation is now considered as a major pathogenic process in PD and offers several targets for preventing α-syn toxicity [74]. While perhaps premature, several clinical trials

focusing on α-syn in PD have been initiated (Table 3), and numerous additional approaches - often reflecting collaboration between industry and academia - are moving forward.

Increasing protein clearance—Given the relationship between α-syn burden and pathology, the obvious first move would be to reduce its expression, through. two strategies: (i) reducing the synthesis or (ii) increasing the clearance. Silencing of *SNCA* in adult animals using small hairpin RNA leads to contrasting results: degeneration [75, 76] and inflammation activation [77] have been reported in rodent models while no effects were reported in squirrel monkey [78]. Several explanations could underlie the apparent inconsistency, including the fact that experiments involved different animal species (rodent *vs.* primates) and may be dependent upon the extent and/or duration of α-syn deficiency: i.e. after partial and temporary α-syn reduction in adult RNAi-treated animals versus lifelong ablation of α-syn in mutant mice. One another possibility is that toxic RNA silencing effects might result from saturation of endogenous RNAi machinery by high RNAi levels, leading to interference of miRNA processing.

Increasing α-syn degradation by enhancing proteasomal or lysosomal activity represents another therapeutic possibility. Recently, 1-[1-(4-fluorophenyl)-2,5-dimethylpyrrol-3-yl]-2-pyrrolidin-1-ylethanone, IU1 (a small molecule identified in a high-throughput screening) was shown to enhance proteasomal function associated with increased clearance of tau [79]. This new molecular area should be considered in future studies for PD. As discussed above, α-syn is degraded under pathological conditions by the ALP and relevant to PD, α-syn-mediated impairment of CMA activates macroautophagy *in vitro* [80]. Several studies have reported successful neuroprotection with strategies aiming at increasing autophagy in *in vitro* and *in vivo* models of PD [57, 81-83] (Table 4). For instance, overexpression of the transcription factor EB [82], LAMP2a [83] or Beclin-1 [81] were able to provide neuroprotection in rat models based on the overexpression of human α-syn or in a transgenic mouse model of PD [81-83]. Most of the *in vivo* work is based, however, on viral-mediated overexpression calling for further demonstration in complementary models (Box 1).

Small autophagy-activating molecules, such as rapamycin, hold promise for rapid translation to patients. This compound is a FDA-approved macrolide that inhibits the activity of the mammalian target of rapamycin (mTOR). It efficiently enhances autophagy *in vivo* [57, 84]. However, long-term use of rapamycin is associated with off-target effects (such as interstitial pneumonitis, high levels of triglycerides, reduced wound healing and anemia – some of them are attributed to its immunosuppressant properties that might increase the risk for cancer), which may preclude its chronic use for PD [85]. The disaccharide trehalose is a mTOR-independent activator of autophagy, which also attenuated neuropathology abnormalities in several models of neurodegenerative disorders including PD [86-88]. The exact connection between trehalose and autophagy as well as the mechanisms underlying its neuroprotective effect remain unknown. Two conclusions can however be drawn from the studies with autophagy-activating molecules [81-83]. First, identification of specific and safe compounds boosting specifically the ALP could be a successful strategy. Second, in light of the recent success in terms of safety and tolerability with lentiviral- and AAV-based approaches [89, 90], gene therapy designed to enhance UPS and lysosomal function should be considered.

Acting on α-syn post-translational modifications—Another strategy is to dampen the PTMs associated with the pathological forms of α-syn. Selecting the most suitable PTM remains challenging in light of the conflicting results obtained with the still popular α-syn phosphorylation at S129. While hyperphosphorylation of α-syn is present in the brain of PD patients, its levels are very low [43]. Furthermore, overexpression of a kinase known to phosphorylate at S129, polo-like kinase 2 (PLK2) or its yeast ortholog *CDC5*, is neuroprotective in several *in vitro,* yeast, nematode and rat models of PD [91]. At odds with these studies, Lee and colleagues reported that pharmacological activation of phosphoprotein phosphatase 2 (PP2A) dephosphorylates α-syn and decreases the α-syn burden in a mouse transgenic model of PD [92]. Moreover, overexpression of G-protein-coupled receptor kinase 6, another α-syn kinase, accelerates degeneration in a rat model of PD [93] and preventing phosphorylation at S129 prevents neurotoxicity, while increasing the numbers of large inclusion bodies in transgenic flies [94]. Further studies are therefore needed to completely unravel the pathological significance of S129-phosphorylation and to define its relevance for therapeutic intervention. Regarding the truncated forms of α-syn, recent results suggest that the overexpression of a calpain-specific inhibitor reduces α-syn pathology in the p.A30P mouse transgenic model [95]. Surprisingly, the opposite, e.g. calpain activity enhancement, did not worsen α-syn pathology [95]. Again, additional studies on both postmortem tissues and animal models are needed to elucidate the relevance of truncated α-syn in PD pathogenesis, identify the relevant proteases, and thereby determine the validity of targeting α-syn cleaving proteases for treatment of PD.

Targeting α-syn aggregation—Inhibiting α-syn aggregation remains an extremely attractive target for drug development. Several groups have focused on the disaggregation pathway, *ad hoc* for this purpose. In yeast, this pathway is composed of several molecular chaperones, in particular heat-shock proteins (Hsp) 40 and 70. Hsp40 and 70 are found in LB [96] and overexpression or pharmacological activation of Hsp70 protects from α-syn toxicity in *in vitro* and fly models of PD, notably through a decrease in oligomers [96-98]. Since oligomeric forms are the suspected toxic species, formation of stable fibrils might be an interesting strategy to prevent cell death. An intense research effort is required to fully understand the toxicity of α-syn oligomers. Identifying aggregation inhibitors from compound library screens should be moved to the forefront. Only few have been developed so far and all were reported to efficiently provide neuroprotection; these include *in vitro* agents, such as EGCG [99] and *in vivo* agents, such as anle138b [100] (3-(1,3-benzodioxol-5-yl)-5-(3- bromophenyl)-1*H*-pyrazole), CLR01 [101] and a prolyl oligopeptidase inhibitor, KYP2047 [102, 103] (Table 4). Anle138b has shown neuroprotective properties in prion-infected mice, in two neurotoxic PD models and one genetic model of PD [100, 104]. The combination of these models, sharing similarities with the various aspects of the human condition, represents a singular validation plan that deserves further examination (Box 1). These encouraging results stress the need for a better understanding of α-syn aggregation and represent a potentially fruitful series of targets for therapeutic development.

A currently “hot” strategy is the use of antibodies that target α-syn, similar to what has been tried during the last decade in the AD field. Several groups have reported neuroprotection after passive (based on the use of antibodies against the protein) or active (vaccination-based

approach using full-length protein or short peptides) immunization in transgenic mouse models of PD [105-107]. These reports have led pharmaceutical companies such as Roche to start a phase I clinical trial based on the use of a monoclonal antibody directed against α-syn (PRX002, initially developed by Elan Pharmaceuticals, patent#US7910333 – see Table 3 for NCT number). Active immunization with Affitope PD01 (Affiris, patent #WO2009103105) was found safe in a first pilot study in 32 PD patients (see Table 4 **and** http://www.affiris.at/press_releases/PD01A_MJFF_E.pdf). PD03, from Affitope, will soon be evaluated in patients with PD within the European SYMPATH consortium (http://www.sympath-project.eu/DL/sympath_factsheet_EN.pdf). Both studies include several exploratory efficacy outcome measures. Although passive and active immunization approaches are fascinating, several key questions remain to be answered. First, most studies were performed in transgenic mice in which human α-syn expression is restricted to the brain (PDGFß- and Thy1-A30P-synuclein transgenic mice), while the majority of α-syn is found on the membrane of red blood cells [108]. It is therefore important to investigate in early clinical studies how antibodies react with peripheral α-syn and whether unbound antibodies could gain access to the brain compartment at sufficient levels. Second, α-syn is a cytosolic protein and LB are intraneuronal inclusions. How antibodies would recognize the intracellular protein and promote its intracellular degradation is unknown but the strategy might halt transcellular α-syn propagation. Surprisingly, passive immunization against α-syn activate autophagy [106]. Antibodies might thus trigger non-selective autophagy, thus leading to the clearance of α-syn through a non α-syn-related mechanism. Indeed autophagy might be unselectively activated as part of an innate immune response against pathogens, here the presence of antibodies [109]; thus leading to the clearance of α-syn through a non-α-syn-related mechanism. The use of intrabodies, that are gene-engineered antibodies specifically built for acting intracellularly, might be more specific [110]. Finally, the development of conformational antibodies, i.e. antibodies recognizing the structure of the peptide not simply the sequence, might be an interesting way to target specific oligomers. In this regard, a recent study using the pffs-based model of PD demonstrated that immunotherapy with antibodies specifically targeting misfolded α-syn is blocking the entrance and propagation of α-syn in neurons, and prevents the development of neuropathological abnormalities in the brain [111]. Altogether strategies aiming at decreasing α-syn aggregation either by disaggregation, stabilizing the amyloid pathway or immunization might be of therapeutic interest for PD. However, one must consider that the precise α-syn species to target remains unclear and that there is a theoretical concern that some α-syn species might be protective and that their removal could accelerate the disease process.

Additional strategies—In light of recent data suggesting spreading properties of α-syn, a better knowledge of the underlying mechanisms might provide therapeutic opportunities. First, as discussed above, nucleation appears to be a critical step in α-syn aggregate formation and therefore the identification of the structure of these “seeds” would be a huge asset. The two key components of spreading are secretion and uptake. Several pathways seem to be involved in α-syn transfer from cell to cell, including endocytosis, direct membrane penetration, trans-synaptic dissemination, exosome-mediated transfer and receptor-dependent uptake (reviewed elsewhere [2]).

Lessons learned from clinical trials in AD—Two humanised monoclonal anti-amyloid β (Aβ) plaque antibodies were ineffective in halting cognitive decline after 72 weeks (bapineuzumab) and 78 weeks (solanezumab) of treatment.[114 and 115] Although these clinical trials did not reach their primary endpoints, a decrease in Aβ plaques was identified by PET.[116] An explanation might be that therapeutic strategies for Alzheimer"s disease should take into account both tau and Aβ deposits because the density of cortical tau aggregates correlates with cognitive decline.[117] A second explanation might be that patients had Alzheimer"s disease that was too advanced to hope for recovery, underlining the importance of testing neuroprotective strategies at an early stage. Roughly 25% of clinically diagnosed patients enrolled in the two trials were amyloid negative, as established by PET imaging or CSF Aβ levels. This finding underscores the need for an objective diagnostic biomarker for selection of patients with Parkinson"s disease: a tracer for α-synuclein pathology will be crucial to further drug development. This finding also raises a major conceptual question: can we ultimately reverse α-synuclein lesions in Parkinson"s disease? At least one study[118] has provided positive results. Although no overt neurodegeneration was reported in the mouse model of dementia with Lewy bodies used in this study, suppression of α-synuclein expression decreased the already established synucleinopathy.[118] This strategy should be now tested in a model that leads to frank neurodegeneration (panel).

How to translate preclinical findings to clinical trials in PD patients?—Primary outcomes of previous disease-modification or neuroprotection trials in PD were based on clinical measures. Owing to the slow rate of progression of motor symptoms in PD, observed differences between treatment groups were small and a matter of considerable debate despite innovative concepts such as the "delayed-start" design [1]. Therefore, efforts to improve study design including the development of objective surrogate markers will be critical for future success.

It was recently suggested that MSA and idiopathic REM sleep behavioral disorder (iRBD) may be good models for proof-of-concept studies with compounds targeting α-syn [117]. MSA is a rapidly progressing disorder leading to severe motor disability within a few years [118]. The fast deterioration of MSA clinical outcomes increases the sensitivity to change over time and should allow detecting disease-modifying effects more rapidly than in PD. Clinical trial duration should therefore be shorter in MSA than in PD, and this is a strong advantage when developing a drug, especially in the early stage. Crucial milestones have been reached for successfully conducting clinical intervention trials in MSA patients and survival may be used as primary outcome [117]. Thirty percent of patients with iRBD develop a defined synucleinopathy at 3 years, rising to 66% at 7.5 years. Stratification with prodromal markers of PD (e.g. deficits in olfaction, color vision and cognition, as well as autonomic dysfunction and subtle motor impairment) further increases the risk of conversion up to 65% at 3 years. Thus, the conversion to defined synucleinopathy may be used in stratified cohorts of iRBD patients for testing compounds very early in the neurodegenerative process.

Concluding remarks

α-syn bears an unquestionable role in PD pathogenesis. Experimental research supports several therapeutic strategies to combat α-syn toxicity with promising preclinical results. However, an understanding of clinicopathologic relationship of various α-syn forms in PD patients is just emerging and needs to be strengthened further. Additionally, we need to establish biomarkers (imaging, biochemical, genomic) of α-syn pathology in live patients and their progression over time in order to conduct biomarker-aided clinical trials of novel therapeutics. The current pipeline of interventions targeting α-syn appears to be wide-ranging but with the usual amount of risk associated with novel approaches. In conclusion, we believe that targeting α-syn for treating PD seems to be a suitable direction to adopt but further preclinical studies are needed to de-risk therapeutic targets and trials.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

1. Meissner WG, et al. Priorities in Parkinson"s disease research. Nat Rev Drug Discov. 2011; 10:377–93. [PubMed: 21532567]
2. Lashuel HA, Overk CR, Oueslati A, Masliah E. The many faces of alpha-synuclein: from structure and toxicity to therapeutic target. Nat Rev Neurosci. 2013; 14:38–48. [PubMed: 23254192]
3. Jankovic J. Parkinson"s disease: clinical features and diagnosis. J Neurol Neurosurg Psychiatry. 2008; 79:368–76. [PubMed: 18344392]
4. Polymeropoulos MH, et al. Mutation in the alpha-synuclein gene identified in families with Parkinson"s disease. Science. 1997; 276:2045–7. [PubMed: 9197268]
5. Spillantini MG, et al. Alpha-synuclein in Lewy bodies. Nature. 1997; 388:839–40. [PubMed: 9278044]
6. Goedert M, Spillantini MG, Del Tredici K, Braak H. 100 years of Lewy pathology. Nat Rev Neurol. 2013; 9:13–24. [PubMed: 23183883]
7. Ueda K, et al. Molecular cloning of cDNA encoding an unrecognized component of amyloid in Alzheimer disease. Proc Natl Acad Sci U S A. 1993; 90:11282–6. [PubMed: 8248242]
8. Weinreb PH, Zhen W, Poon AW, Conway KA, Lansbury PT Jr. NACP, a protein implicated in Alzheimer"s disease and learning, is natively unfolded. Biochemistry. 1996; 35:13709–15. [PubMed: 8901511]
9. Iwai A, et al. The precursor protein of non-A beta component of Alzheimer"s disease amyloid is a presynaptic protein of the central nervous system. Neuron. 1995; 14:467–75. [PubMed: 7857654]
10. Lesage S, et al. G51D alpha-synuclein mutation causes a novel parkinsonian-pyramidal syndrome. Ann Neurol. 2013; 73:459–71. [PubMed: 23526723]
11. Kruger R, et al. Ala30Pro mutation in the gene encoding alpha-synuclein in Parkinson"s disease. Nat Genet. 1998; 18:106–8. [PubMed: 9462735]

12. Zarranz JJ, et al. The new mutation, E46K, of alpha-synuclein causes Parkinson and Lewy body dementia. Ann Neurol. 2004; 55:164–73. [PubMed: 14755719]
13. Appel-Cresswell S, et al. Alpha-synuclein p.H50Q, a novel pathogenic mutation for Parkinson"s disease. Mov Disord. 2013; 28:811–3. [PubMed: 23457019]
14. Proukakis C, et al. A novel alpha-synuclein missense mutation in Parkinson disease. Neurology. 2013; 80:1062–4. [PubMed: 23427326]
15. Pasanen P, et al. A novel alpha-synuclein mutation A53E associated with atypical multiple system atrophy and Parkinson"s disease-type pathology. Neurobiol Aging. 2014
16. Seidel K, et al. First appraisal of brain pathology owing to A30P mutant alpha-synuclein. Ann Neurol. 2010; 67:684–9. [PubMed: 20437567]
17. Kotzbauer PT, et al. Fibrillization of alpha-synuclein and tau in familial Parkinson"s disease caused by the A53T alpha-synuclein mutation. Exp Neurol. 2004; 187:279–88. [PubMed: 15144854]
18. Chartier-Harlin MC, et al. Alpha-synuclein locus duplication as a cause of familial Parkinson"s disease. Lancet. 2004; 364:1167–9. [PubMed: 15451224]
19. Singleton AB, et al. alpha-Synuclein locus triplication causes Parkinson"s disease. Science. 2003; 302:841. [PubMed: 14593171]
20. Bendor JT, Logan TP, Edwards RH. The function of alpha-synuclein. Neuron. 2013; 79:1044–66. [PubMed: 24050397]
21. Rochet JC, Hay BA, Guo M. Molecular insights into Parkinson"s disease. Prog Mol Biol Transl Sci. 2012; 107:125–88. [PubMed: 22482450]
22. Giasson BI, Murray IV, Trojanowski JQ, Lee VM. A hydrophobic stretch of 12 amino acid residues in the middle of alpha-synuclein is essential for filament assembly. J Biol Chem. 2001; 276:2380–6. [PubMed: 11060312]
23. Wang W, et al. A soluble alpha-synuclein construct forms a dynamic tetramer. Proc Natl Acad Sci U S A. 2011; 108:17797–802. [PubMed: 22006323]
24. Bartels T, Choi JG, Selkoe DJ. alpha-Synuclein occurs physiologically as a helically folded tetramer that resists aggregation. Nature. 2011; 477:107–10. [PubMed: 21841800]
25. Gould N, et al. Evidence of native alpha-synuclein conformers in the human brain. J Biol Chem. 2014; 289:7929–34. [PubMed: 24474688]
26. Fauvet B, et al. alpha-Synuclein in central nervous system and from erythrocytes, mammalian cells, and Escherichia coli exists predominantly as disordered monomer. J Biol Chem. 2012; 287:15345–64. [PubMed: 22315227]
27. Conway KA, Harper JD, Lansbury PT. Accelerated in vitro fibril formation by a mutant alpha-synuclein linked to early-onset Parkinson disease. Nat Med. 1998; 4:1318–20. [PubMed: 9809558]
28. Conway KA, et al. Acceleration of oligomerization, not fibrillization, is a shared property of both alpha-synuclein mutations linked to early-onset Parkinson"s disease: implications for pathogenesis and therapy. Proc Natl Acad Sci U S A. 2000; 97:571–6. [PubMed: 10639120]
29. Karpinar DP, et al. Pre-fibrillar alpha-synuclein variants with impaired beta-structure increase neurotoxicity in Parkinson"s disease models. EMBO J. 2009; 28:3256–68. [PubMed: 19745811]
30. Winner B, et al. In vivo demonstration that alpha-synuclein oligomers are toxic. Proc Natl Acad Sci U S A. 2011; 108:4194–9. [PubMed: 21325059]
31. Cremades N, et al. Direct observation of the interconversion of normal and toxic forms of alpha-synuclein. Cell. 2012; 149:1048–59. [PubMed: 22632969]
32. Danzer KM, et al. Different species of alpha-synuclein oligomers induce calcium influx and seeding. J Neurosci. 2007; 27:9220–32. [PubMed: 17715357]
33. Tanaka M, et al. Aggresomes formed by alpha-synuclein and synphilin-1 are cytoprotective. J Biol Chem. 2004; 279:4625–31. [PubMed: 14627698]
34. Volles MJ, Lansbury PT Jr. Vesicle permeabilization by protofibrillar alpha-synuclein is sensitive to Parkinson"s disease-linked mutations and occurs by a pore-like mechanism. Biochemistry. 2002; 41:4595–602. [PubMed: 11926821]
35. Quist A, et al. Amyloid ion channels: a common structural link for protein-misfolding disease. Proc Natl Acad Sci U S A. 2005; 102:10427–32. [PubMed: 16020533]

36. van Rooijen BD, Claessens MM, Subramaniam V. Membrane Permeabilization by Oligomeric alpha-Synuclein: In Search of the Mechanism. PLoS One. 2010; 5:e14292. [PubMed: 21179192]
37. Freeman D, et al. Alpha-synuclein induces lysosomal rupture and cathepsin dependent reactive oxygen species following endocytosis. PLoS One. 2013; 8:e62143. [PubMed: 23634225]
38. Choi BK, et al. Large alpha-synuclein oligomers inhibit neuronal SNARE-mediated vesicle docking. Proc Natl Acad Sci U S A. 2013; 110:4087–92. [PubMed: 23431141]
39. Okochi M, et al. Constitutive phosphorylation of the Parkinson"s disease associated alpha-synuclein. J Biol Chem. 2000; 275:390–7. [PubMed: 10617630]
40. Fujiwara H, et al. alpha-Synuclein is phosphorylated in synucleinopathy lesions. Nat Cell Biol. 2002; 4:160–4. [PubMed: 11813001]
41. Anderson JP, et al. Phosphorylation of Ser-129 is the dominant pathological modification of alpha-synuclein in familial and sporadic Lewy body disease. J Biol Chem. 2006; 281:29739–52. [PubMed: 16847063]
42. Paleologou KE, et al. Phosphorylation at Ser-129 but not the phosphomimics S129E/D inhibits the fibrillation of alpha-synuclein. J Biol Chem. 2008; 283:16895–905. [PubMed: 18343814]
43. Kellie JF, et al. Quantitative measurement of intact alpha-synuclein proteoforms from post-mortem control and Parkinson"s disease brain tissue by intact protein mass spectrometry. Sci Rep. 2014; 4:5797. [PubMed: 25052239]
44. Giasson BI, et al. Oxidative damage linked to neurodegeneration by selective alpha-synuclein nitration in synucleinopathy lesions. Science. 2000; 290:985–9. [PubMed: 11062131]
45. Conway KA, Rochet JC, Bieganski RM, Lansbury PT Jr. Kinetic stabilization of the alpha-synuclein protofibril by a dopamine-alpha-synuclein adduct. Science. 2001; 294:1346–9. [PubMed: 11701929]
46. Norris EH, Giasson BI, Ischiropoulos H, Lee VM. Effects of oxidative and nitrative challenges on alpha-synuclein fibrillogenesis involve distinct mechanisms of protein modifications. J Biol Chem. 2003; 278:27230–40. [PubMed: 12857790]
47. Yamin G, Uversky VN, Fink AL. Nitration inhibits fibrillation of human alpha-synuclein in vitro by formation of soluble oligomers. FEBS Lett. 2003; 542:147–52. [PubMed: 12729915]
48. Periquet M, Fulga T, Myllykangas L, Schlossmacher MG, Feany MB. Aggregated alpha-synuclein mediates dopaminergic neurotoxicity in vivo. J Neurosci. 2007; 27:3338–46. [PubMed: 17376994]
49. Ulusoy A, Febbraro F, Jensen PH, Kirik D, Romero-Ramos M. Co-expression of C-terminal truncated alpha-synuclein enhances full-length alpha-synuclein-induced pathology. Eur J Neurosci. 2010; 32:409–22. [PubMed: 20704592]
50. Bennett MC, et al. Degradation of alpha-synuclein by proteasome. J Biol Chem. 1999; 274:33855–8. [PubMed: 10567343]
51. Webb JL, Ravikumar B, Atkins J, Skepper JN, Rubinsztein DC. Alpha-Synuclein is degraded by both autophagy and the proteasome. J Biol Chem. 2003; 278:25009–13. [PubMed: 12719433]
52. Cuervo AM, Stefanis L, Fredenburg R, Lansbury PT, Sulzer D. Impaired degradation of mutant alpha-synuclein by chaperone-mediated autophagy. Science. 2004; 305:1292–5. [PubMed: 15333840]
53. Martinez-Vicente M, et al. Dopamine-modified alpha-synuclein blocks chaperone-mediated autophagy. J Clin Invest. 2008; 118:777–88. [PubMed: 18172548]
54. Winslow AR, et al. alpha-Synuclein impairs macroautophagy: implications for Parkinson"s disease. J Cell Biol. 2010; 190:1023–37. [PubMed: 20855506]
55. Tanik SA, Schultheiss CE, Volpicelli-Daley LA, Brunden KR, Lee VM. Lewy body-like alpha-synuclein aggregates resist degradation and impair macroautophagy. J Biol Chem. 2013; 288:15194–210. [PubMed: 23532841]
56. Ebrahimi-Fakhari D, et al. Distinct roles in vivo for the ubiquitin-proteasome system and the autophagy-lysosomal pathway in the degradation of alpha-synuclein. J Neurosci. 2011; 31:14508–20. [PubMed: 21994367]
57. Dehay B, et al. Pathogenic lysosomal depletion in Parkinson"s disease. J Neurosci. 2010; 30:12535–44. [PubMed: 20844148]

58. Chu Y, Dodiya H, Aebischer P, Olanow CW, Kordower JH. Alterations in lysosomal and proteasomal markers in Parkinson"s disease: relationship to alpha-synuclein inclusions. Neurobiol Dis. 2009; 35:385–98. [PubMed: 19505575]
59. Bellucci A, et al. Induction of the unfolded protein response by alpha-synuclein in experimental models of Parkinson"s disease. J Neurochem. 2011; 116:588–605. [PubMed: 21166675]
60. Guardia-Laguarta C, et al. alpha-Synuclein is localized to mitochondria-associated ER membranes. J Neurosci. 2014; 34:249–59. [PubMed: 24381286]
61. Mattson MP, Magnus T. Ageing and neuronal vulnerability. Nat Rev Neurosci. 2006; 7:278–94. [PubMed: 16552414]
62. Kordower JH, Chu Y, Hauser RA, Freeman TB, Olanow CW. Lewy body-like pathology in long-term embryonic nigral transplants in Parkinson"s disease. Nat Med. 2008; 14:504–6. [PubMed: 18391962]
63. Li JY, et al. Lewy bodies in grafted neurons in subjects with Parkinson"s disease suggest host-to-graft disease propagation. Nat Med. 2008; 14:501–3. [PubMed: 18391963]
64. Mendez I, et al. Dopamine neurons implanted into people with Parkinson"s disease survive without pathology for 14 years. Nat Med. 2008; 14:507–9. [PubMed: 18391961]
65. Braak H, et al. Staging of brain pathology related to sporadic Parkinson"s disease. Neurobiol Aging. 2003; 24:197–211. [PubMed: 12498954]
66. Recasens A, et al. Lewy body extracts from Parkinson disease brains trigger alpha-synuclein pathology and neurodegeneration in mice and monkeys. Ann Neurol. 2014; 75:351–62. [PubMed: 24243558]
67. Luk KC, et al. Pathological alpha-synuclein transmission initiates Parkinson-like neurodegeneration in nontransgenic mice. Science. 2012; 338:949–53. [PubMed: 23161999]
68. Recasens A, Dehay B. Alpha-synuclein spreading in Parkinson"s disease. Front Neuroanat. 2014; 8:159. [PubMed: 25565982]
69. Bousset L, et al. Structural and functional characterization of two alpha-synuclein strains. Nat Commun. 2013; 4:2575. [PubMed: 24108358]
70. Kim C, et al. Neuron-released oligomeric alpha-synuclein is an endogenous agonist of TLR2 for paracrine activation of microglia. Nat Commun. 2013; 4:1562. [PubMed: 23463005]
71. Hirsch EC, Hunot S. Neuroinflammation in Parkinson"s disease: a target for neuroprotection? Lancet Neurol. 2009; 8:382–97. [PubMed: 19296921]
72. Benner EJ, et al. Nitrated alpha-synuclein immunity accelerates degeneration of nigral dopaminergic neurons. PLoS One. 2008; 3:e1376. [PubMed: 18167537]
73. Brochard V, et al. Infiltration of CD4+ lymphocytes into the brain contributes to neurodegeneration in a mouse model of Parkinson disease. J Clin Invest. 2009; 119:182–92. [PubMed: 19104149]
74. Schapira AH, Olanow CW, Greenamyre JT, Bezard E. Slowing of neurodegeneration in Parkinson"s disease and Huntington"s disease: future therapeutic perspectives. Lancet. 2014; 384:545–55. [PubMed: 24954676]
75. Gorbatyuk OS, et al. In vivo RNAi-mediated alpha-synuclein silencing induces nigrostriatal degeneration. Mol Ther. 2010; 18:1450–7. [PubMed: 20551914]
76. Khodr CE, et al. An alpha-synuclein AAV gene silencing vector ameliorates a behavioral deficit in a rat model of Parkinson"s disease, but displays toxicity in dopamine neurons. Brain Res. 2011; 1395:94–107. [PubMed: 21565333]
77. Khodr CE, Becerra A, Han Y, Bohn MC. Targeting alpha-synuclein with a microRNA-embedded silencing vector in the rat substantia nigra: positive and negative effects. Brain Res. 2014; 1550:47–60. [PubMed: 24463035]
78. McCormack AL, et al. Alpha-synuclein suppression by targeted small interfering RNA in the primate substantia nigra. PLoS One. 2010; 5:e12122. [PubMed: 20711464]
79. Lee BH, et al. Enhancement of proteasome activity by a small-molecule inhibitor of USP14. Nature. 2010; 467:179–84. [PubMed: 20829789]
80. Xilouri M, Vogiatzi T, Vekrellis K, Park D, Stefanis L. Abberant alpha-synuclein confers toxicity to neurons in part through inhibition of chaperone-mediated autophagy. PLoS One. 2009; 4:e5515. [PubMed: 19436756]

81. Spencer B, et al. Beclin 1 gene transfer activates autophagy and ameliorates the neurodegenerative pathology in alpha-synuclein models of Parkinson"s and Lewy body diseases. J Neurosci. 2009; 29:13578–88. [PubMed: 19864570]

82. Decressac M, et al. TFEB-mediated autophagy rescues midbrain dopamine neurons from alpha-synuclein toxicity. Proc Natl Acad Sci U S A. 2013; 110:E1817–26. [PubMed: 23610405]

83. Xilouri M, et al. Boosting chaperone-mediated autophagy in vivo mitigates alpha-synuclein-induced neurodegeneration. Brain. 2013; 136:2130–46. [PubMed: 23757764]

84. Malagelada C, Jin ZH, Jackson-Lewis V, Przedborski S, Greene LA. Rapamycin protects against neuron death in in vitro and in vivo models of Parkinson"s disease. J Neurosci. 2010; 30:1166–75. [PubMed: 20089925]

85. Bove J, Martinez-Vicente M, Vila M. Fighting neurodegeneration with rapamycin: mechanistic insights. Nat Rev Neurosci. 2011; 12:437–52. [PubMed: 21772323]

86. Sarkar S, Davies JE, Huang Z, Tunnacliffe A, Rubinsztein DC. Trehalose, a novel mTOR-independent autophagy enhancer, accelerates the clearance of mutant huntingtin and alpha-synuclein. J Biol Chem. 2007; 282:5641–52. [PubMed: 17182613]

87. Rodriguez-Navarro JA, et al. Trehalose ameliorates dopaminergic and tau pathology in parkin deleted/tau overexpressing mice through autophagy activation. Neurobiol Dis. 2010; 39:423–38. [PubMed: 20546895]

88. Castillo K, et al. Trehalose delays the progression of amyotrophic lateral sclerosis by enhancing autophagy in motoneurons. Autophagy. 2013; 9:1308–20. [PubMed: 23851366]

89. Marks WJ Jr. et al. Gene delivery of AAV2-neurturin for Parkinson"s disease: a double-blind, randomised, controlled trial. Lancet Neurol. 2010; 9:1164–72. [PubMed: 20970382]

90. Palfi S, et al. Long-term safety and tolerability of ProSavin, a lentiviral vector-based gene therapy for Parkinson"s disease: a dose escalation, open-label, phase 1/2 trial. Lancet. 2014; 383:1138–46. [PubMed: 24412048]

91. Oueslati A, Schneider BL, Aebischer P, Lashuel HA. Polo-like kinase 2 regulates selective autophagic alpha-synuclein clearance and suppresses its toxicity in vivo. Proc Natl Acad Sci U S A. 2013; 110:E3945–54. [PubMed: 23983262]

92. Lee KW, et al. Enhanced phosphatase activity attenuates alpha-synucleinopathy in a mouse model. J Neurosci. 2011; 31:6963–71. [PubMed: 21562258]

93. Sato H, et al. Authentically phosphorylated alpha-synuclein at Ser129 accelerates neurodegeneration in a rat model of familial Parkinson"s disease. J Neurosci. 2011; 31:16884–94. [PubMed: 22090514]

94. Chen L, Feany MB. Alpha-synuclein phosphorylation controls neurotoxicity and inclusion formation in a Drosophila model of Parkinson disease. Nat Neurosci. 2005; 8:657–63. [PubMed: 15834418]

95. Diepenbroek M, et al. Overexpression of the calpain-specific inhibitor calpastatin reduces human alpha-Synuclein processing, aggregation and synaptic impairment in [A30P]alphaSyn transgenic mice. Hum Mol Genet. 2014

96. Auluck PK, Chan HY, Trojanowski JQ, Lee VM, Bonini NM. Chaperone suppression of alpha-synuclein toxicity in a Drosophila model for Parkinson"s disease. Science. 2002; 295:865–8. [PubMed: 11823645]

97. Klucken J, Shin Y, Masliah E, Hyman BT, McLean PJ. Hsp70 Reduces alpha-Synuclein Aggregation and Toxicity. J Biol Chem. 2004; 279:25497–502. [PubMed: 15044495]

98. Auluck PK, Bonini NM. Pharmacological prevention of Parkinson disease in Drosophila. Nat Med. 2002; 8:1185–6. [PubMed: 12411925]

99. Bieschke J, et al. EGCG remodels mature alpha-synuclein and amyloid-beta fibrils and reduces cellular toxicity. Proc Natl Acad Sci U S A. 2010; 107:7710–5. [PubMed: 20385841]

100. Wagner J, et al. Anle138b: a novel oligomer modulator for disease-modifying therapy of neurodegenerative diseases such as prion and Parkinson"s disease. Acta Neuropathol. 2013; 125:795–813. [PubMed: 23604588]

101. Prabhudesai S, et al. A novel "molecular tweezer" inhibitor of alpha-synuclein neurotoxicity in vitro and in vivo. Neurotherapeutics. 2012; 9:464–76. [PubMed: 22373667]

102. Savolainen MH, et al. The beneficial effect of a prolyl oligopeptidase inhibitor, KYP-2047, on alpha-synuclein clearance and autophagy in A30P transgenic mouse. Neurobiol Dis. 2014; 68C: 1–15.
103. Myohanen TT, et al. A prolyl oligopeptidase inhibitor, KYP-2047, reduces alpha-synuclein protein levels and aggregates in cellular and animal models of Parkinson"s disease. Br J Pharmacol. 2012; 166:1097–113. [PubMed: 22233220]
104. Levin J, et al. The oligomer modulator anle138b inhibits disease progression in a Parkinson mouse model even with treatment started after disease onset. Acta Neuropathol. 2014; 127:779–80. [PubMed: 24615514]
105. Bae EJ, et al. Antibody-aided clearance of extracellular alpha-synuclein prevents cell-to-cell aggregate transmission. J Neurosci. 2012; 32:13454–69. [PubMed: 23015436]
106. Masliah E, et al. Passive immunization reduces behavioral and neuropathological deficits in an alpha-synuclein transgenic model of Lewy body disease. PLoS One. 2011; 6:e19338. [PubMed: 21559417]
107. Mandler M, et al. Next-generation active immunization approach for synucleinopathies: implications for Parkinson"s disease clinical trials. Acta Neuropathol. 2014; 127:861–79. [PubMed: 24525765]
108. Fuchs J, et al. Genetic variability in the SNCA gene influences alpha-synuclein levels in the blood and brain. FASEB J. 2008; 22:1327–34. [PubMed: 18162487]
109. Pareja ME, Colombo MI. Autophagic clearance of bacterial pathogens: molecular recognition of intracellular microorganisms. Front Cell Infect Microbiol. 2013; 3:54. [PubMed: 24137567]
110. Zhou C, Przedborski S. Intrabody and Parkinson"s disease. Biochim Biophys Acta. 2009; 1792:634–42. [PubMed: 18834937]
111. Tran HT, et al. Alpha-synuclein immunotherapy blocks uptake and templated propagation of misfolded alpha-synuclein and neurodegeneration. Cell Rep. 2014; 7:2054–65. [PubMed: 24931606]
112. Salloway S, et al. Two phase 3 trials of bapineuzumab in mild-to-moderate Alzheimer"s disease. N Engl J Med. 2014; 370:322–33. [PubMed: 24450891]
113. Doody RS, et al. Phase 3 trials of solanezumab for mild-to-moderate Alzheimer"s disease. N Engl J Med. 2014; 370:311–21. [PubMed: 24450890]
114. Rinne JO, et al. 11C-PiB PET assessment of change in fibrillar amyloid-beta load in patients with Alzheimer"s disease treated with bapineuzumab: a phase 2, double-blind, placebo-controlled, ascending-dose study. Lancet Neurol. 2010; 9:363–72. [PubMed: 20189881]
115. Lee VM, Brunden KR, Hutton M, Trojanowski JQ. Developing therapeutic approaches to tau, selected kinases, and related neuronal protein targets. Cold Spring Harb Perspect Med. 2011; 1:a006437. [PubMed: 22229117]
116. Lim Y, et al. alpha-Syn suppression reverses synaptic and memory defects in a mouse model of dementia with Lewy bodies. J Neurosci. 2011; 31:10076–87. [PubMed: 21734300]
117. Fernagut PO, et al. Multiple system atrophy: a prototypical synucleinopathy for disease-modifying therapeutic strategies. Neurobiol Dis. 2014; 67:133–9. [PubMed: 24727096]
118. Fanciulli A, Wenning GK. Multiple-system atrophy. N Engl J Med. 2015; 372:249–63. [PubMed: 25587949]
119. Bezard E. Neuroprotection for Parkinson"s disease: a call for clinically driven experimental design. Lancet Neurol. 2003; 2:393. [PubMed: 12849115]
120. Bezard E, Yue Z, Kirik D, Spillantini MG. Animal models of Parkinson"s disease: limits and relevance to neuroprotection studies. Mov Disord. 2013; 28:61–70. [PubMed: 22753348]

Textbox 1

Clinically driven experimental designs for target validation and drug development

Currently, there are many α-syn-based models of PD developed from simple to more complex organisms. They contributed to a better understanding of the function of α-syn, etiology and pathogenesis of PD. While none of them reproduce the full pathology, all share some similarities with the disease. In this regard, the use of a set of complementary models seems mandatory to fully validate a new therapeutic target.

As illustrated in Table 4, most preclinical studies use an experimental design in which the therapeutic compound is administered either prophylactically or concomitantly with the induction of pathology. This raises the question of the relevance regarding the progressive nature of PD. Patients can be treated only after the onset of symptoms and diagnosis (usually around 30%-50% of dopaminergic cell loss). Hence, there is a crucial need for clinically driven experiments to validate drug candidates. Ideally, these experiments should fulfill four criteria [119, 120]. First, the chosen model should represent the progressive nature of PD, thus avoiding the use of acute toxic models. One cannot ignore the progressive nature of the disease, as some drugs, such as minocycline, were deleterious only in chronic models. Second, the therapeutic compound should be administered after symptom onset and once degeneration has started. Third, the therapeutic compound should be tested in complementary animal models (pathogenic and etiologic). Fourth, final proof of efficacy should be obtained in the non-human primate model of PD, as neuronal physiology is knowingly different between primate and rodents. Although it is impossible to remove all doubts before testing a drug in early phases in patients, our ethical obligation is to use the most relevant preclinical validation method. Hence, to increase the chance of successful clinical trials, we must use a combination of relevant models associated with a clinically driven experimental design to test neuroprotective compounds in preclinical investigations.

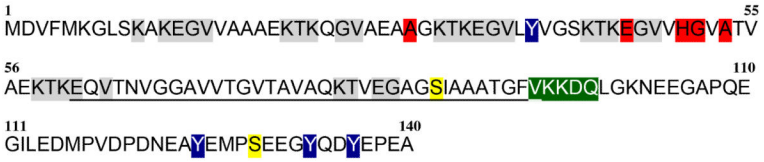

Figure 1. Primary structure of human α-synuclein (UniProtKB/Swiss-Prot: P37840)

Clinical Mutations (A53T, A30P, E46K, H50Q, G51D, A53E) are indicated in red. Amphipathic N-terminal region contains six imperfect lysine-rich highly conserved motif repeats (KTKEGV), which involve in binding of lipids, marked in grey. Central hydrophobic region contains non-amyloid beta component (NAC) sequence from residue 61 to 95 is underlined. Two major phosphorylation sites (Ser87 and Ser129) are colored in yellow. CMA recognition sites are marked in green. Nitration sites (Y39, Y125, Y133, Y136) are colored in blue.

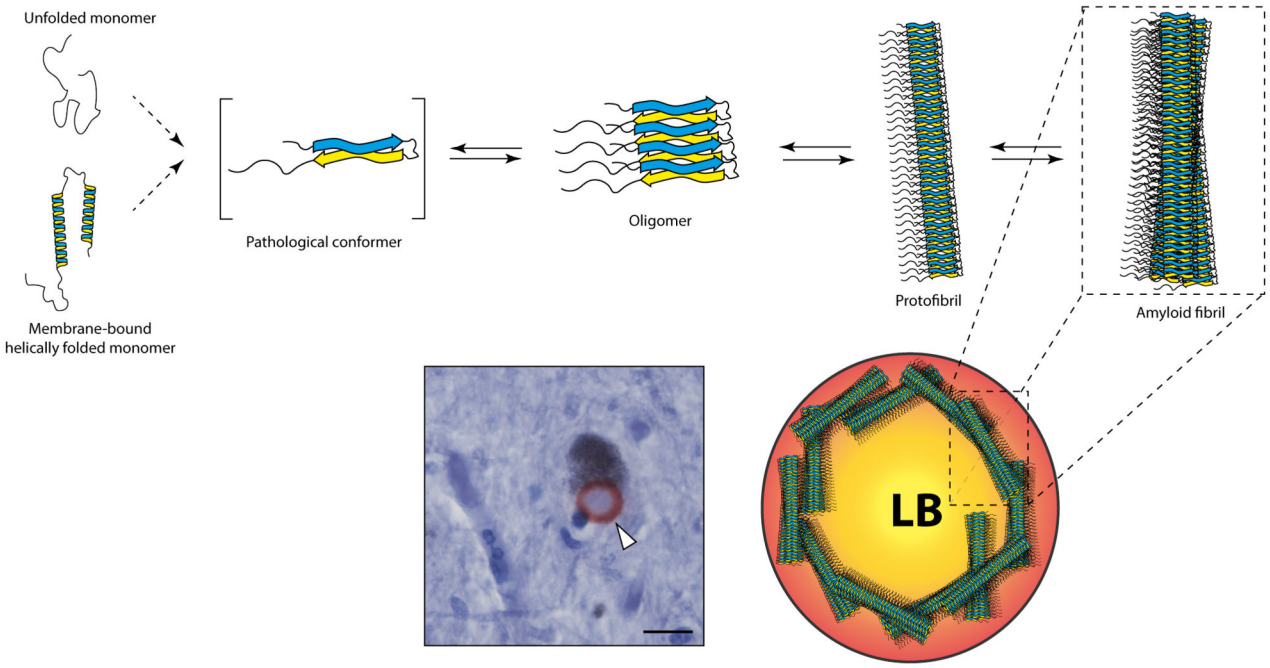

Figure 2. Schematic summary of α-synuclein aggregation pathway

The panel shows that α-syn exists under various conformational shapes. α-Syn exists as at least two structural isoforms: a natively unfolded monomer and a helix-rich membrane-bound form. Both isoforms may undergo dramatic structural changes resulting in the formation of β-sheet rich assemblies. From in vitro studies, it is clear that α-syn behaves in a dynamic equilibrium where monomer can aggregate first into several types of small oligomeric species that can be stabilized by β-sheet interactions and then into higher molecular weight insoluble protofibrils and may polymerize into amyloidogenic fibrils resembly those found in Lewy Body (LB). However, the mechanism governing the fundamental conformational change of normal monomeric α-syn to a pathological, disease-associated form, remains unknown. Photomicrograph illustrates one synuclein stained-mesencephalic LB (in red) in a neuromelanin-positive neuron from sporadic PD patient indicated by the white arrow. Scale bar: 5μm.

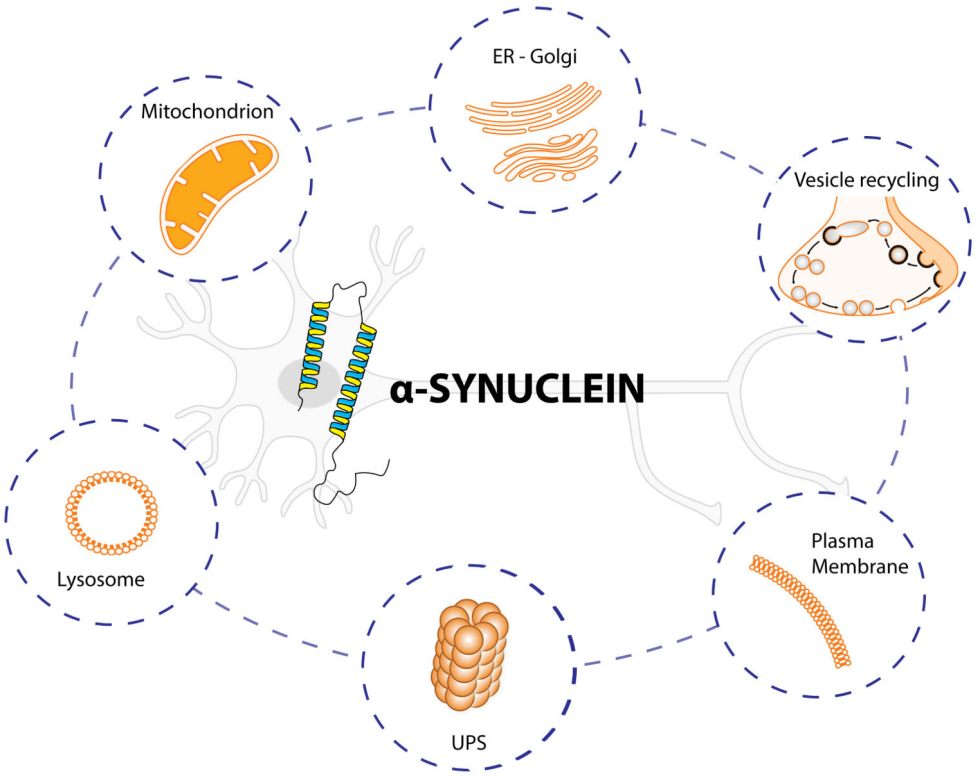

Figure 3. Schematic summary of established interactions between α-synuclein and cellular components

The figure highlights six different intracellular pathways affected by α-synuclein (α-syn). The protein α-syn is enriched at the pre-synaptic terminals of almost all types of neurons in the brain, where it participates in the vesicle recycling, thereby modulating synaptic function. α-syn can be degraded by the ubiquitin-proteasome system (UPS) and inside the lysosomes. α-syn interacts strongly with membranes, such as plasma membrane and mitochondrion. If misfolded, α-syn forms distinct structures that are prone to aggregation, first into oligomers, then into larger structures. It is now believed that α-syn oligomers are the toxic form that may impair basic neuronal processes, such as ER-Golgi trafficking, lysosome and UPS functions, reduced mitochondrial activity and alter the plasma membrane through the pore/perforations that can dysregulate calcium and cation homeostasis.

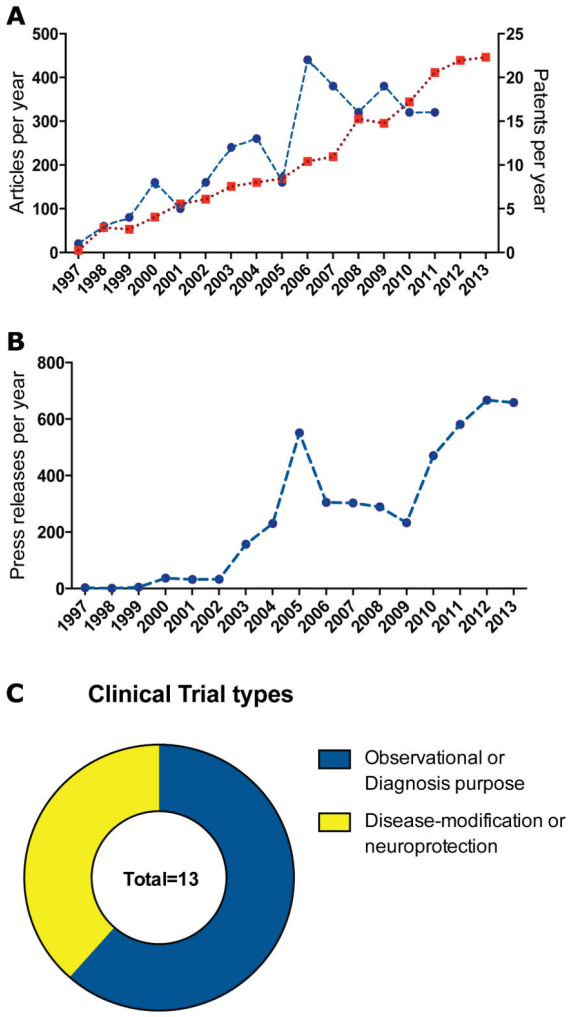

Figure 4. Analysis of α-synuclein-related publications, patents and press releases per year

(A) The emergence of the α-syn research field as measured by the number of scientific articles per year (in red) is accompanied by patents deposition (in blue). No gap between the two curves is observed. The patent (blue) line stops in 2011 since we used the priority date, which is closely equivalent to the deposition date (the patent will only become public 18 months after deposit). Literature search was conducted in PubMed, as well in Scopus® (Elsevier) and Web of Science® (Thomson Reuters) databases with MeSH terms and/or keywords in title, and abstract fields. Between 2127 and 5026 documents were retrieved on the subject depending on the database and the type of documents (article, review, notes, proceedings…) from 1997 to 2013. The patent analysis was run in the world wide collection of INPADOC family patents using Orbit® (Questel) patent research platform through a boolean search combining keywords in different topic field (title, abstract & claims) and International Patent sub-class Code (IPC) A61P-025/16 for antiparkinsonian drugs. All subsequent analyses were performed on patent family, i.e. a set of patent applications with the same priority date in different countries related to the same invention. Since 1997, and up to December 31th, 2013, we identified 176 patent families filed worldwide (in blue). **(B)** Press release in large media per year. The curve tendency fits with scientific publications and

patent deposits. For the analysis, we used a collection of 3576 documents retrieved from Scopus for which we extracted citations count and compared to trends of press releases in large media by searching Factiva® (Dow Jones) database. Two peaks emerge, a first in 2005 with the post-genomic revolution and the interference RNA technology breakthrough, and a second one in 2010 with the support of the Michael J. Fox Foundation (MJFF) for candidate compounds and the launch of clinical trials (e.g. Affiris). **(C)** Of 1313 studies in the field of PD, only 13 open studies are associated with the "synuclein" keyword and 8 are actually biomarker studies. We divided clinical trials in two categories, observational or for diagnosis purpose (n=8) and disease modifying or neuroprotective strategies (n=5). Data were collected from the Food and Drug Administration's clinical trials database (clinicaltrials.gov). Clinical trials, which were terminated, completed or of which the status was unknown were excluded. Abbreviations: NIH: national institute of health; α-syn: α-synuclein.

Table 1

Patents focusing on α-synuclein that have not been announced in the pipeline

α-syn: α-synuclein; PD: Parkinson's disease; AD: Alzheimer's disease; MSA: Multiple System Atrophy; DLB: Dementia with Lewy Body; *GBA*: Glucocerebrosidase gene name; ß-syn: ß-synuclein; Aß: amyloid-beta; siRNA: small interfering RNA; miRNA: microRNA; RNAi : RNA interference.

Patent publication #	Year of publication	Assignee	Main claims
WO2013127918	2013	**Pharnext** *(Issy Les Moulineaux, France)*	Use of acamprosate, baclofen, cinalcet, mexiletine, sulfasoxazol, torasemide to delay PD
WO2013063516	2013	**Neotop Bioscience** *(Dublin, Ireland)*	New antibody panel for PD and other Lewy Body diseases
WO2013020368	2013	**Hong Kong university** *(Hong Kong, China)*	*Rhodiola rosea* extract (e.g. rosavin) as an α-syn oligomerization inhibitor
WO2012170899	2012	**Prothera** *(Reno, USA)*	Protease or peptidase (prolyl oligopeptidase) for PD patients with *GBA* mutations
WO2012068405	2012	**ISIS Pharmaceuticals** *(Carlsbad, USA)*	New oligonucleotide library to decrase α-syn expression
WO2011107544	2011	**Dr. Rentschlzer Holding** *(Laupheim, Deutchland)*	Antibody library targeting α-syn for PD and synucleinopathies
WO2011056222	2011	**University of Pittsburg** *(Pittsburg, USA)*	Anti-protein aggregate compound
WO2010129791	2010	**University Of Medicine & Dentistry of New Jersey** *(Somerset, USA)*	Use of miRNA to decrase α-syn expression
WO2010103515	2010	**Tel Aviv University** *(Tel-Aviv, Israel)*	Use of ß-syn derived peptides to decrease α-syn aggregation
WO2010094090	2010	**Katholleke University Leuven** *(Leuven, Belgium)* & **University of Graz** *(Gras, Austria)*	Sodium/hydrogen exchange type-1 transport system inhibitors against α-syn toxicity
WO2010060073	2010	**Tel Aviv University** *(Tel-Aviv, Israel)*	Bacteriophage-based therapy for synucleinopathy
WO2010037135	2010	**University Of California** *(Oakland, USA)* & **Rosalind Franklin University of Medicine** *(Chicago, USA)*	Increasing clearance of protein aggregates by virus-mediated expression of chimeric polypeptide
WO2009086306	2009	**Whitehead Institute for Biomedical Research** *(Cambridge, USA)*	List of target genes involved in α-syn toxicity
WO2009020624	2009	**University of Alabama** *(Tuscaloosa, USA)*	SURF, SEC22 and Acyl CoA Oxidase alterations and modulations for PD
WO2008157425	2008	**University of California** *(Oakland, USA)*	Use of polypeptide to slow protein aggregation in PD, AD, MSA, DLB
WO2008002465	2008	**Feinstein Institute For Medical Research** *(Manhasset, USA)*	Guanylhydrazone compounds to prevent Aß and α-syn aggregation
WO2007135426	2007	**Isis Innovation** *(Oxford, GB)*	Agents (RNAi, siRNA,DNA, ribozyme) to decrease α-syn expression levels
WO2006124892	2006	**Whitehead Institute for Biomedical Research** *(Cambridge, USA)*	List of target genes involved in α-syn toxicity
WO2006091964	2006	**University of Alabama** *(Tuscaloosa, USA)*	Methods for identification and targeting of genes involved in α-syn aggregation
WO2006073734	2006	**Whitehead Institute for Biomedical Research** *(Cambridge, USA)* & **University of Missouri** *(Missouri, USA)*	List of target genes involved in α-syn toxicity
WO2006039253	2006	**Children"s Memorial Hospital** *(Chicago, USA)*	siRNA to decrease α-syn expression

Lancet Neurol. Author manuscript; available in PMC 2017 January 06.

Patent publication #	Year of publication	Assignee	Main claims
WO0020020	2000	**University of California** *(La Jolla, USA)*	Use of ß-syn as a dominant-negative effect to decrease α-syn aggregation

Table 2

List of compounds targeting either directly or indirectly α-synuclein which are in the pipeline but not yet translated to clinical trials

Compounds listed here are still in preclinical development. For clarity purpose, we excluded the two compounds that are already in clinical development: PD01 and PRX002. Targets, mechanism of action, PCT numbers are indicated when information was available. α-syn: α-synuclein; GCase: glucocerebrosidase; GAIM: general amyloid interaction motif; IgG: immunoglobulin G; PTC: patent cooperation treaty.

Drug Name	Sponsor	Target Name	Mechanisms of action	PCT or priority patent
AT-3375	**Amicus Therapeutics** *(Cranbury, USA)*	**GCase**	Pharmacological chaperone that increases GCase enzyme activity	WO2007137237, WO2007150064, WO2010118282
PD-0805	**BioArctic Neuroscience** *(Stockholm, Sweden)*	α-syn	Antibody against “toxic” forms of α-syn	WO2009133521
EDP-32	**BioLineRx** *(Jerusalem, Israel)*	α-syn	Aggregation inhibitor	WO2010103515
NI-202	**Neurimmune Therapeutics** *(Schlieren, Switzerland)*	α-syn	Antibody	WO2010069603
NPT-088	**NeuroPhage** *(Cambridge, USA)*	α-syn, Tau, Aß	Second generation of NPT-001; human IgG1 fusion protein containing GAIM	
NPT-001	**NeuroPhage** *(Cambridge, USA)*	α-syn, Tau, Aß	Bacteriophage M13 capsid with GAIM recognizing and remodeling misfolded protein aggregates	
NLF-1233	**nLife Therapeutics** *(Granada, Spain)*	α-syn	RNAi	WO2014064257, WO2011131693
PBT-434	**Prana Biotechnology** *(Parkville, Australia)*	*Unspecified*	Metal (iron) attenuating compound	
USP14 inhibitors	**Proteostasis Therapeutics** *(Cambridge, USA)*	Ubiquitin specific peptidase 14	Proteasome activity enhancer	
ReS9-S7	**reMYND** *(Leuven, Belgium)*	*Unspecified*	Facilitates α-syn degradation	
ReS12-S	**reMYND** *(Leuven, Belgium)*	α-syn	Chemical, synthetic	WO2009019295
SIG-1012	**Signum Biosciences** *(Monmouth Junction, USA)*	Protein phosphatase 2 (formerly 2A)	α-syn phosphorylation enhancer	
G2 PD	**TauRx Pharmaceuticals** *(Singapore, Republic of Singapore)*	*Unspecified*	Compound targeting misfolded α-syn	

Table 3

Active clinical trials testing new emergent drugs and/or targets for PD treatment

PD: Parkinson's disease; α-syn: α-synuclein; MSA: multiple system atrophy: UMSARS-ME: motor examination (ME) of the Unified MSA Rating Scale

ClinicalTrials.gov identifiers	Title	Primary outcome measures
NCT01885494	AFF008E: Observational Phase 1b Follow-up Extension Study for Patients With PD After Immunization With **AFFITOPE® PD01A**	Tolerability and Safety
NCT02046434	**Phenylbutyrate** As a Treatment for Abnormal Accumulation of Brain Protein in PD	Levels of α-syn in blood plasma
NCT02095171	Single Ascending Dose Study of **PRX002** in Healthy Subjects	Safety, tolerability and pharmacokinetics
NCT02157714	Multiple Ascending Dose Study of **PRX002** in Patients With PD	Safety, tolerability and pharmacokinetics
NCT02008721	Progression Rate of MSA Under **EGCG** Supplementation as Anti-Aggregation-Approach	UMSARS – ME

Table 4

Effects of gene expression strategies and pharmacological compounds in experimental in vivo models of Parkinson's disease

In regards to experimental design, preclinical studies can be sorted in four groups depending on the lag time between the induction of the pathology and the administration of the neuroprotective compound. The therapeutic compound can be administered: (I) prophylactically, (II) concomitantly or (III) after onset of symptoms. The fourth group (IV) is based on the use of transgenic animals. In most cases, the therapeutic compound is administered to adult transgenic animals, although rare examples of delivery after onset of symptoms exist (Anle138b for instance). AAV, adeno-associated virus; CMA, chaperone-mediated autophagy; TFEB, transcription factor EB; LAMP2a, lysosome-associated membrane protein 2 type a; α-syn, α-synuclein; mTOR, mammalian target of rapamycin; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; PLK2, polo-like kinase 2; PP2A, Protein phosphatase-2A ; Thy1, THYmocyte differentiation antigen 1 ; Hsp70, heat shock protein 70; Hsp104, heat shock protein 104; LV, lentivirus ; Snca, synuclein gene nomenclature ; PDGFβ, platelet-derived growth factor-β; Pffs: α-syn preformed fibrils.

Target	Mechanism of action	Animal Model	Main outcome	Experimental design	References
Autophagic gene expression					
TFEB	Enhancement of lysosomal biogenesis	Rat AAV2/6 - α-syn	Neuroprotection Decreases α-syn pathology	II	123
LAMP2a	Boosting of CMA function	Rat AAV2/6 - α-syn	Neuroprotection Decreases α-syn pathology	II	124
Beclin 1	Enhancement of autophagy	Mice PDGFß- α-syn	Decreases α-syn pathology	IV	122
		Rat AAV2/6 - α-syn	Neuroprotection Decrease α-syn pathology	II	123
Autophagy-enhancing drug					
Rapamycin	Inhibition of mTOR	Mice - MPTP	Neuroprotection	I	87
Temsirolimus	Rapamycin analog	Rat AAV2/6 - α-syn	Neuroprotection Decreases α-syn pathology	III	123
Trehalose	Enhancement of autophagy	Mice Parkin deleted / tau overexpressing	Neuroprotection	IV	127
Modulation of protein phosphorylation					
PLK2	Phosphorylation of α-syn at S129	Rat AAV2/6 - α-syn	Neuroprotection Decreases α-syn pathology	II	134
PP2A *(Pharmacological activation)*	Dephosphorylation of α-syn at S129	Mice Thy1- α-syn	Restores motor behaviour Decreases α-syn pathology and glial activation	IV	136
Inhibition of α-syn truncation					
Calpastatin *(Overexpression of inhibitor)*	Truncation inhibitor	Mice Thy1-A30P- α-syn	Decreases α-syn pathology and glial activation	IV	138

Target	Mechanism of action	Animal Model	Main outcome	Experimental design	References
Disaggregation of α-syn					
Hsp70 *(Overexpression)*	Molecular chaperone	Fly- α-syn	Neuroprotection	IV	139
Hsp70 *(Pharmacological activation)*	Molecular chaperone	Fly - α-syn	Neuroprotection	IV	141
HSP104	Molecular chaperone	Rat LV-A30P - α-syn	Neuroprotection Decreases α-syn pathology	II	63
Anle138b	Oligomer modulator	Mice Rotenone Thy1 - A30P - α-syn	Decreases behavioral impairments Decreases α-syn pathology Increases in disease-free survival	IV - III	144 & 148
CRL01	Molecular tweezer	Zebrafish - α-syn	Increases survival Decreases α-syn pathology	IV	145
KYP-2047	Prolyl oligopeptidase inhibitor	Mice Thy1-A30P - α-syn $Snca^{tm(A30P)}$	Fiber protection Decreases α-syn pathology	IV	146 & 147
α-syn antibodies					
9E4	Passive immunization	Mice PDGFß - α-syn	Decreases α-syn pathology	IV	149
AFFITOPE® technology	Active immunization	Mice Thy1 - α-syn PDGFß - α-syn	Improves behavioral impairments Decreases α-syn pathology and glial activation Induce inflammatory response	IV	150
Syn303	Passive immunization	Mice Pffs induced degeneration	Reduces motor dysfunction Decreases dopaminergic cell loss and α-syn pathology	II - III	157