

Review

Recent Advances in Autophagy and Immunotherapy for the Clearance of Aggregated α -Synuclein in Parkinson's Disease

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ABSTRACT: Parkinson's disease is a neurodegenerative condition characterized by the accumulation of misfolded and aggregated α -synuclein in Lewy bodies and neurites. These protein aggregates contribute to neurodegeneration and motor symptoms such as bradykinesia, rigidity, and tremor. While the ubiquitin-proteasome system degrades soluble α -synuclein, aggregated and oligomeric forms are primarily cleared via the autophagy-lysosomal pathway. Mutations of the *SNCA* gene exacerbate α -synuclein aggregation and significantly impair its clearance, highlighting the importance of targeting toxic α -synuclein species. Strategies such as promoting autophagosome formation via 5'-AMP-activated protein kinase (AMPK) and mechanistic target of rapamycin complex 1 (mTORC1) or facilitating autophagosome maturation via RAB7-a member of the RAS oncogene family-and related effectors, have shown promise in enhancing autophagy and reducing α -synuclein pathology. Pharmacological agents such as rapamycin, trehalose, and nilotinib have demonstrated preclinical efficacy in enhancing α -synuclein clearance and alleviating disease features. Concurrently, immunotherapy approaches, including passive and active immunization, aim to enhance the immune system's ability to recognize and eliminate toxic α -synuclein species. Emerging strategies such as peptide-based therapies aim to inhibit aggregation or promote degradation of α -synuclein. At the same time, nanotechnology enables the targeted delivery of therapeutic agents across the blood-brain barrier with improved efficiency. Additionally, novel AUTOTAC (autophagy-targeting chimera) platforms offer a precision strategy to tag α -synuclein for autophagic degradation. This review explores many advances in autophagy-mediated aggregated α -synuclein clearance, emphasizing its potential as a therapeutic strategy to address the limitations of current symptomatic treatments and slow the progression of Parkinson's disease.

Key words: alpha-synuclein, Parkinson's disease, small molecule, peptide, immunotherapy

INTRODUCTION

Parkinson's disease (PD) is a progressive neurodegenerative disease (NDD) characterized by the accumulation of misfolded and aggregated α -synuclein in Lewy bodies and neurites, which are pathological hallmarks of the disease. α -Synuclein aggregation disrupts cellular homeostasis, contributing to neurodegeneration

and clinical manifestations such as bradykinesia, rigidity, and tremor [1,2]. Under normal conditions, soluble α -synuclein is primarily degraded by the ubiquitin-proteasome system (UPS), while α -synuclein aggregates and oligomers are preferentially cleared via the autophagy-lysosomal pathway (ALP). However, structural constraints of the proteasome and the inability of chaperones to efficiently unfold α -synuclein oligomers

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for degradation indicate the critical role of autophagy in mitigating the burden of aggregated α -synuclein [3-6]. Mutations and polymorphisms in the *SNCA* gene are implicated in familial and sporadic forms of PD, further exacerbating α -synuclein aggregation. *SNCA* gene duplication and point mutations, such as A53T, A30P, and E46K, increase the propensity of α -synuclein to misfold and form toxic oligomers and fibrils. These genetic alterations not only enhance α -synuclein aggregation but also disrupt normal pathways of cellular trafficking and degradation, leading to its accumulation [7,8]. The interplay between genetic mutations and impaired protein clearance underscores the potential of targeting aggregation-prone α -synuclein species through pathways such as autophagy to mitigate PD progression. Aggregated α -synuclein triggers autophagy, a crucial cellular breakdown pathway. During autophagy, cytosolic components, including aggregated proteins, are sequestered into double-membrane vesicles called autophagosomes. These then merge with lysosomes for degradation. Promoting autophagy may help remove α -synuclein aggregates and slow disease progression in PD [9].

Autophagy is a carefully regulated cellular process that involves the degradation and recycling of damaged organelles, misfolded proteins, and other cytoplasmic components. Cellular stress or nutrient deprivation initiates the process by activating or inhibiting upstream regulators, such as 5'-AMP-activated protein kinase (AMPK) and mechanistic target of rapamycin complex 1 (mTORC1), leading to activation of the Unc-51 like autophagy activating kinase 1 (ULK1) complex. This triggers the formation of a membrane structure known as the phagophore, which is guided by the Beclin-1-VPS34 complex and other autophagy-related (ATG) proteins. The phagophore then expands, wrapping around its cargo to create a double-membraned vesicle known as the autophagosome. Two primary strategies have been explored to enhance autophagy for the clearance of α -synuclein aggregates. The first involves increasing autophagosome formation by targeting the upstream regulators of autophagy initiation [10]. Pharmacological agents such as metformin, rapamycin, nilotinib, and nicotinamide have been shown to effectively promote autophagy initiation. The second strategy focuses on facilitating autophagosome maturation. In these processes, the autophagosome fuses with the lysosome to form an autolysosome, allowing for the degradation and recycling of its contents. Once the autophagosome is fully formed and has enclosed damaged organelles or misfolded proteins, it is transported along microtubules toward the perinuclear region, where lysosomes are concentrated. This transport is regulated by motor proteins and small GTPases, such as Rab7. The fusion

between the autophagosome and the lysosome is mediated by specific tethering factors and SNARE proteins, including Syntaxin 17 (STX17), synaptosome-associated protein 29 (SNAP29), and vesicle-associated membrane protein 8 (VAMP8). Upon fusion, lysosomal hydrolases degrade both the inner autophagosomal membrane and its cargo, breaking them down into basic biomolecules like amino acids, lipids, and sugars. These are then transported back into the cytoplasm for reuse. Small GTPases, such as RAB7 of the RAS oncogene family, and their effectors, such as FYVE and coiled-coil domain autophagy adaptor 1 (FYCO1), play critical roles in regulating these processes. Their potential for reducing α -synuclein pathology has been demonstrated in cellular and animal models of PD [11,12]. Additionally, misfolded or aggregated α -synuclein acts as a damage-associated molecular pattern (DAMP) recognized by innate immune receptors on microglia, such as Toll-like receptors (TLRs), particularly TLR2 and TLR4. Activation of these receptors triggers inflammatory signaling cascades, including the NF- κ B pathway, promoting the release of pro-inflammatory cytokines, chemokines, and reactive oxygen species (ROS). In parallel, α -synuclein-induced neuroinflammation can engage receptor-interacting protein kinases RIPK1 and RIPK3, key mediators of necroptosis, a regulated form of inflammatory cell death. Activation of RIPK1/3 signaling contributes to microglial activation, cytokine release, and neuronal cell death, thereby amplifying the cycle of neurodegeneration and chronic inflammation characteristic of synucleinopathies [13,14] (Fig. 1).

Current pharmacological treatments for PD, including levodopa, monoamine oxidase B (MAOB) inhibitors, and dopamine agonists, provide symptomatic relief but are often associated with significant side effects, such as motor fluctuations and cognitive impairments. In this context, autophagy-enhancing agents, such as trehalose, isorhynchophylline, latrepirdine, and nilotinib, offer a promising therapeutic approach. These agents have demonstrated efficacy in promoting the degradation of mutant α -synuclein and alleviating pathological features in preclinical models of PD. Therefore, this review aims to explore recent advances in the clearance of α -synuclein aggregates through autophagy in depth. By examining molecular mechanisms, therapeutic strategies, and emerging agents targeting the ALP, we aim to highlight the potential of autophagy modulators as innovative treatments for PD.

Defective autophagy in PD

Autophagy is a crucial process for maintaining cellular homeostasis and promoting cell differentiation and survival by degrading cytoplasmic elements, including

proteins, lipids, nucleic acids, and damaged mitochondria. In lysosome-mediated autophagy, or macroautophagy, defective proteins or organelles are engulfed by a double-layered autophagosome, which then fuses with a lysosome to form an autophagolysosome. Autophagosome formation is primarily regulated by the inhibition and activation of mTOR and AMPK, respectively [15,16]. α -synuclein has a complex

bidirectional relationship with autophagy; dysfunctional autophagy can lead to α -synuclein aggregation, and α -synuclein aggregation can interfere with autophagy, impairing the clearance of α -synuclein and other cellular substances [17,18]. Several genes associated with PD have a detrimental effect on autophagy, either by impairing autophagosome formation and maturation or lysosome function.

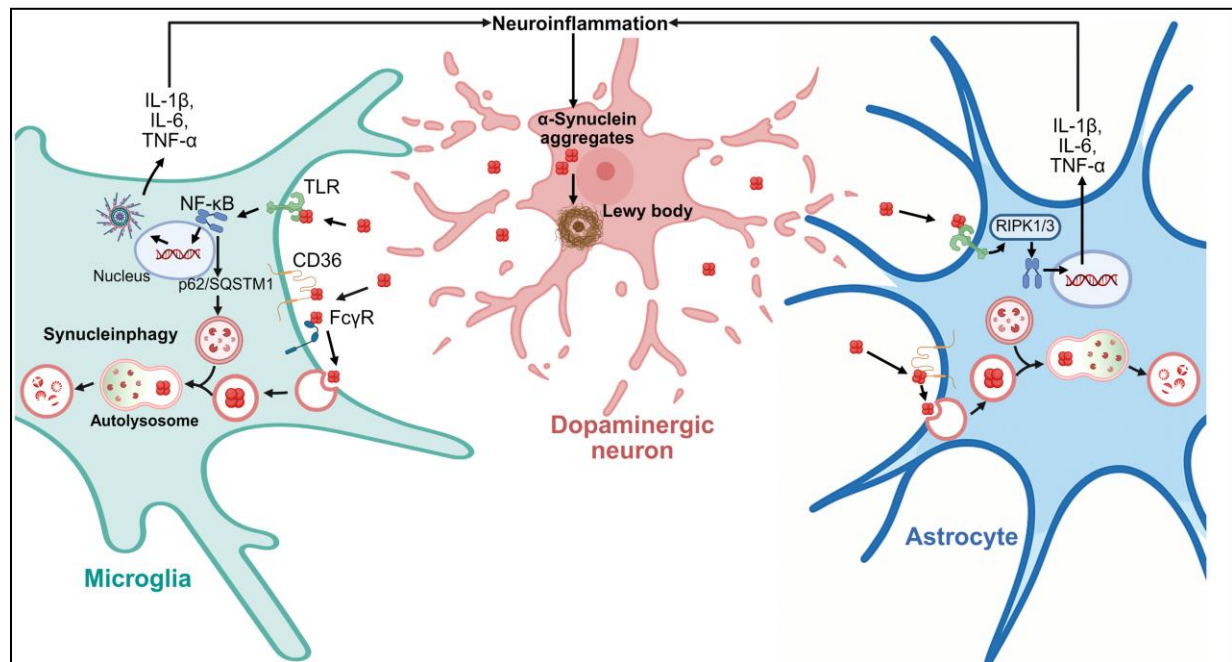


Figure 1. Extracellular α -synuclein: a double-edged signal balancing neuro-inflammation and autophagic clearance.

Fibrillar α -synuclein released from degenerating dopaminergic neurons acts via two parallel mechanisms: (i) it activates TLR2/4-, CD36-, and Fc γ R-dependent pathways, leading to NF- κ B and RIPK1/3 signaling in neighboring microglia and astrocytes, which promotes the secretion of IL-1 β , IL-6, and TNF- α ; and (ii) it is selectively targeted for degradation via p62/SQSTM1-mediated autophagy, in which α -synuclein is sequestered into autophagosomes and subsequently cleared through lysosomal fusion, a process known as 'synucleinophagy'. This balance between pro-inflammatory signaling and degradative clearance shapes the neuroinflammatory milieu and ultimately influences neuronal survival. TLR: Toll-like receptor; NF- κ B: Nuclear factor kappa B; RIPK: Receptor-interacting protein kinase; IL: Interleukin; TNF: Tumor necrosis factor; SQSTM: Sequestosome.

Leucine-rich repeat kinase 2 (*LRRK2*) is associated with both familial and sporadic PD. The function of *LRRK2* is complex, with roles in various cellular processes, particularly neuronal function and maintenance. Mutations in *LRRK2* (G2019S and R1441C) and *Lrrk2* knockdown or knockout in mice impair autophagy in PD. *LRRK2* inhibits autophagy by blocking phagophore biogenesis and autophagosome formation, leading to deficient autophagosome-lysosome fusion [19]. In chaperone-mediated autophagy (CMA), lysosome-associated membrane protein 2 splice variant A (LAMP2A) translocates substrates into the lysosome via the formation of a translocation complex by oligomerization. Mutated *LRRK2* binds strongly to LAMP2A and inhibits its oligomerization, preventing the

formation of the necessary translocation complex and blocking lysosomal degradation of α -synuclein and other substrates. This impaired autophagy leads to α -synuclein accumulation in neurons [20].

The glucocerebrosidase-beta (*GBA*) gene encodes the GCase lysosomal enzyme that hydrolyzes glucosylceramide (GlcCer) to ceramide and glucose, essential for maintaining cellular homeostasis and proper lysosomal function. An estimated 5%-10% of patients with PD have *GBA* mutations, with N370S and L444P the most common, making them major genetic risk factors [21]. Mutations in *GBA* cause its loss of function, leading to deficient GCase activity and GlcCer accumulation in the lysosome. Consequently, α -synuclein cannot enter the lysosome for degradation, increasing its aggregation [20].

However, α -synuclein aggregation can also impair GCase activity by interfering with GCase trafficking from the endoplasmic reticulum to the lysosome via the Golgi body [22].

Membrane expansion for autophagosome formation is a complex and poorly understood process, for which the autophagy-related protein ATG9 is crucial. α -synuclein overexpression and aggregation cause the mislocalization of ATG9 by RAB1A, a member of the RAS oncogene family, impairing autophagosome formation [23,16]. Autophagosome-lysosome fusion is another key event in the degradation of unwanted molecules via autophagy. SNAP29 is a vesicle SNARE protein that mediates the fusion of autophagosomes with lysosomes. Overexpression of α -synuclein impairs SNAP29-mediated autophagosome-lysosome fusion [24].

Mutations in *SNCA* can cause familial early-onset PD. Studies have shown that mutations such as A30P,

A53T, and E46K result in rapid fibril formation and variations in their structure [25,26]. Both wild-type (WT) and mutated α -synuclein impair the autophagy pathway. Zinc finger with Krüppel-associated box (KRAB) and SCAN domains 3 (ZKSCAN3/ZNF306) belongs to a family of zinc-finger transcription factors that transcriptionally repress autophagy [27]. The α -synuclein^{A30P} mutant is reported to inhibit autophagy by affecting JUN N-terminal kinase (JNK) signaling, thereby enhancing ZKSCAN3 activity [28]. Variations in fibril structure and properties caused by mutations can impact autophagy through diverse mechanisms. It is therefore essential to finely modulate the pathways involved in their clearance. However, few studies have examined the effects of mutations on fibril clearance, warranting further exploration of this topic.

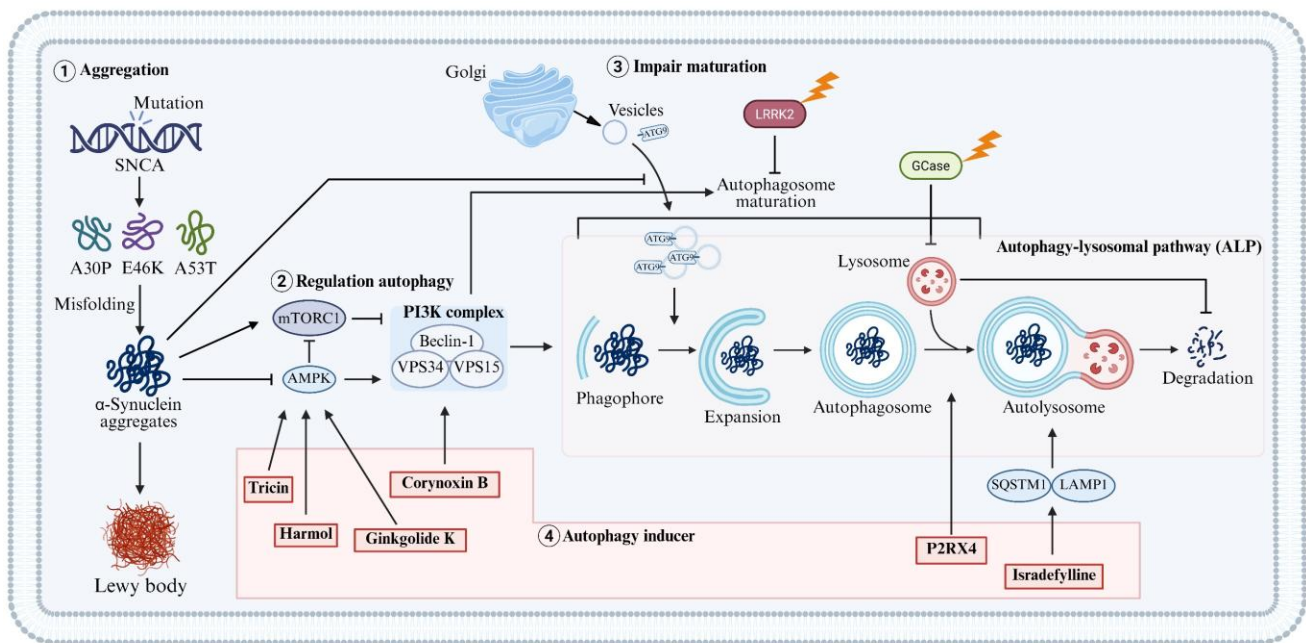


Figure 2. Schematic overview of autophagy-mediated clearance of aggregated α -synuclein in PD. (1) Pathogenic α -synuclein mutations (A30P, E46K, A53T) promote misfolding and aggregation, ultimately leading to Lewy body formation. (2) The diagram illustrates key regulatory components of the autophagy–lysosomal pathway (ALP), including AMPK, mTORC1, and the class III PI3K complex (Beclin-1, VPS34, VPS15), which are critical for phagophore formation and autophagosome maturation. (3) Aggregated α -synuclein disrupts ATG9 vesicle trafficking from the Golgi, impairing phagophore initiation, while dysfunction of LRRK2 and reduced GCase activity independently impair autophagosome maturation and lysosomal degradation, contributing to global autophagic dysfunction. (4) Several small-molecule modulators (Tricin, Ginkgolide K, Corynoxin B, Harmol) and pathways involving P2RX4, SQSTM1, and LAMP1 are shown to restore autophagic flux and promote α -synuclein degradation, potentially alleviating neurodegeneration. PD: Parkinson's disease; AMPK: AMP-activated protein kinase; mTORC1: Mammalian target of rapamycin complex 1; PI3K: Phosphoinositide 3-kinase; GCase: Glucocerebrosidase; VPS: Vacuolar protein-sorting; ATG: Autophagy-related protein; LRRK: Leucine-rich repeat kinase.

Small molecules activate autophagy and eliminate toxic protein aggregates

Autophagy inducers are chemicals or interventions that increase ALP activity, facilitating the degradation and removal of accumulated proteins. These inducers promote

the autophagic breakdown of α -synuclein, preventing harmful aggregation, reducing cellular stress, and protecting susceptible neurons. Advances in the understanding of autophagy mechanisms and the development of tailored autophagy inducers have opened new avenues for therapeutic interventions in PD. This review examines how autophagy inducers can facilitate the clearance of accumulated α -synuclein, including their mechanisms of action, therapeutic efficacy, and implications for PD development.

Recent advances in autophagy-inducing small molecules highlight promising therapeutic avenues for PD. These molecules act through diverse mechanisms to target autophagy-related proteins, lysosomal biogenesis, and CMA activation, providing potential alternatives to conventional symptomatic treatments. Small molecules that modulate autophagy have shown great promise in facilitating the clearance of α -synuclein aggregates through promoting autophagosome formation or enhancing autophagosome maturation and lysosomal degradation (Table 1, Fig. 2).

Table 1. Small-molecule autophagy inducers that help to clear α -synuclein aggregates.

Compound	Study model	Dose	Duration of treatment	Therapeutic effects	Refs.
<i>In Vitro study</i>					
Trehalose, lactulose or, melibiose	α -synuclein-GFP SH-SY5Y cells	100 μ M	8 hours	Increased LC3-II/LC3-I ratio, <i>NRF-2</i> expression, and α -synuclein aggregates were inhibited and cleared.	[32]
Corynoxin B	N2a cells, PC-12 cells	20 mM/L,	6 hours	Enhanced BECN1/VPS34 complex activity, promoted the interaction between BECN1 and HMGB1/2 which induced autophagy for α -synuclein ^{WT} and α -synuclein ^{A53T} aggregate clearance.	[37]
Ginkgolide K	SH-SY5Y cells	30 μ g/mL	24 hours	Activated the LC-II/class-III PI3K pathway and promoted α -synuclein ^{A53T} clearance.	[41]
Maysin	SH-SY5Y cells	5 μ M	24 hours	Reduced RPS6 expression, increased the LC3-II/LC3-I ratio, and enhanced autophagosome formation to clear protein aggregates.	[43]
Tricin	Tet-on α -synuclein ^{A53T} inducible PC-12 cells SH-SY5Y cells	40–100 μ M	24 hours	Activated the AMPK-p70s6K signaling pathway and ATG7-dependent autophagy and enhanced α -synuclein clearance in both cellular models.	[44]
Celastrol	MoDCs	0.25 μ M	1 hour	Upregulated <i>Rab5</i> and <i>Rab7</i> expression, increased <i>Becn1</i> expression, reduced <i>p62</i> expression, and elevated LC3 ratio facilitated the trafficking of α -synuclein towards the lysosome and promoted the clearance of fibrillar proteins.	[47]
Berberine, coptisine, and palmatine	α -synuclein ^{A53T} -GFP SH-SY5Y cells	10 μ M	8 hours	Increased TFEB expression and the LC3-II/LC3-I ratio induced autophagy and inhibited α -synuclein ^{A53T} aggregation.	[48]
GM1 ganglioside	PC-12 (α -synuclein ^{A53T}) cells	40 μ g/mL	24 hours	Phosphorylation of ATG13 and ULK1 enhanced SCNA ^{A53T} degradation.	[49]
Albendazole	SH-SY5Y cells	10 μ M	8 hours	Induced lysosomal clustering through a JIP4/TRPML1-dependent mechanism and autophagy-dependent degradation of Triton-X-insoluble aggregates.	[53]
Mc1568	SH-SY5Y cells	0–10 μ M	2 hours	HDAC4 inhibition induced autophagy.	[59]
AGK2	SH-SY5Y cells	5 mM	24 hours	Increased phosphorylation of P70S6K, decreased total 4EBP1, and clearance of α -synuclein aggregates from the cell enhanced cell survival.	[60]
Harmol	Tet-on α -synuclein ^{A53T}	30 μ M	6-24 hours	Phosphorylation of ULK1 at Ser757 activated the AMPK/mTOR/TFEB	[64]

	inducible PC-12 cells			pathway, promoting autophagy and lysosomal biogenesis.	
Dihydromyricetin (DHM) and salvianolic acid B (Sal B)	H4 neuroglioma cells expressing SynT-aggregation	DHM (10 μ M), Sal B (50 μ M)	16 hours	Increased LAMP1 and LAMP2A levels and degraded α -synuclein aggregates.	[73]
In Vivo study					
Corynoxin B	<i>Prp</i> - α -synuclein α -synuclein ^{A53T} transgenic mice	20 mg/kg, intraperitoneal injection	Daily for one month	Cleared the insoluble or aggregated α -synuclein ^{WT} and α -synuclein ^{A53T} and improved behavioral performances.	[37]
Istradefylline (KW6002)	Male C57BL/6 mice	5 mg/kg/body weight (b.w.), intraperitoneal injection	Every other day for 1 month	Regulating SQSTM1 and LAMP2A expression cleared α -synuclein ^{A53T} protofibrils from the mouse frontal cortex.	[63]
Harmol	α -synuclein ^{A53T} mice	40 mg/kg/b.w., oral gavage	Twice a day, Daily for 1 month	Activated the AMPK/mTOR/TFEB pathway, promoting autophagy leading to the clearance of aggregated protein in substantia nigra	[64]
Ferruginol	Transgenic α -synuclein ^{A53T} C57BL/6 mice	50 or 100 mg/kg/b.w., intraperitoneal injection	Daily for 21 days	Increased BECN1/LC3-I/II ratio attenuated α -synuclein aggregation and altered behavioral deficits.	[65]
HSEA-P1, P2	<i>C. elegans</i> (NL5901 strain)	10 μ g/mL, dropped in nematode growth media agar plate	Every other day for 9 days	Regulated <i>bec-1</i> (BECN1), <i>lgg-1</i> (LC3), and <i>atg-7</i> (ATG7) and degraded α -synuclein aggregates.	[67]
Piperine	<i>Thyl</i> - α -synuclein transgenic mice expressing human α -synuclein	25, 50, and 100 mg/kg/b.w., oral administration	Daily for 6 weeks	Activated P2RX4-mediated autophagy, degraded aggregated α -synuclein <i>in vivo</i>	[72]
Dihydromyricetin (DHM) and salvianolic acid B (Sal B)	Transgenic mice expressing human α -synuclein ^{WT}	10mg/kg/b.w./day, intraperitoneal injection	Daily for 2 weeks	Increased LAMP1 and LAMP2A levels and degraded α -synuclein aggregates.	[73]

Abbreviations: SNCA: Synuclein alpha; α -syn: Alpha synuclein; GFP: Green fluorescence protein; LC3: Microtubule-associated protein 1A/1B-light chain 3; Nrf-2: Nuclear factor erythroid 2-related factor 2; N2a cells: Neuro2a cells (mouse neuroblastoma); PC-12: VPS34: Vacuolar protein-sorting 34; HMGB1/2: High Mobility Group Box 1 and 2 protein; PI3K: Phosphoinositide 3-kinase; AMPK: AMP-activated protein kinase; ATG: Autophagy-related protein; Rab: Ras-associated binding; TFEB: Transcription Factor EB; ULK: Unc-51-like kinase; HDAC: Histone deacetylase; SQSTM: Sequestosome; LAMP: Lysosome-associated membrane protein.

Trehalose, a plant disaccharide, has proven effective against aggregate-prone NDDs [29-31]. Lactulose and melibiose, two indigestible analogs of trehalose, were found to exhibit promising effects of α -synuclein aggregation inhibition and autophagy induction. In human neuroblastoma SH-SY5Y cells, induced expression of SNCA-GFP (green fluorescent protein), combined with the addition of α -synuclein fibrils over six days, reduced the microtubule-associated protein 1 light chain 3 (LC3-II/LC3-I) ratio to 57% ($p < 0.001$) compared to control cells (100%). Meanwhile, treatment with trehalose, lactulose, or melibiose (100 μ M) significantly increased the ratio to 86%–90% ($p = 0.012$ – 0.005) relative to the fibril-treated cells [32]. The primary signaling complex needed for autophagosome formation and maturation is the phosphatidylinositol 3-kinase III (PI3KC3) complex, which consists of vacuolar protein sorting 34 (VPS34), vacuolar protein sorting 15 (VPS15), and beclin 1

(BECN1). The PI3KC3 complex synthesizes phosphatidylinositol 3-phosphate (PI3P); PI3P promotes the recruitment of further autophagic components and encourages the autophagosome's double membrane to expand and the lysosome to fuse with the autophagosome [33-35]. α -synuclein^{WT} and α -synuclein^{A53T} bind to high mobility group box 1 (HMGB1), inhibiting its translocation to the cytosol and thereby preventing its interaction with BECN1, impairing autophagy [36]. Corynoxin B (Cory-B) is an alkaloid from *Uncaria rhynchophylla* (Miq.) Jacks that produced promising results in enhancing α -synuclein clearance from cells. Treating rat pheochromocytoma PC-12 cells with 20 mM/L of Cory-B significantly activated the PI3KC3 complex and increased the interaction between HMGB1/2 and BECN1. In mouse neuroblastoma N2a cells, Cory-B bound directly to HMGB1/2. Treating transgenic mice with 20 mg/kg Cory-B promoted the clearance of α -

synuclein^{WT} and α -synuclein^{A53T} and improved behavioral outcomes [37].

Ginkgolide K, a compound isolated from *Ginkgo biloba* leaves, exhibits neuroprotective activity in PD [38,39]. In a cellular PD model, ginkgolide K induced autophagy through the AMPK/mTOR/ULK1 signaling pathway [40]. In SH-SY5Y cells overexpressing α -synuclein^{A53T}, ginkgolide K at 30 mg/mL cleared mutated α -synuclein from cells by 50-fold through the lipidated light chain 3 (LC3-II)/PI3K pathway [41]. Autophagy was also induced by maysin, a C-glycosyl flavone (rhamnosyl-6-C-(4-ketofucosyl)-5,7,3',4'-tetrahydroxyflavone), isolated from corn silk (*Stigma maydis*). Treating SH-SY5Y cells with 5 mM maysin significantly reduced ribosomal protein S6 (RPS6) phosphorylation—a crucial downstream substrate of the primary suppressive regulator of mTOR [42]—thus inducing autophagy. The increased LC3-I/LC3-II ratio confirmed autophagosome formation [43]. Tricin, a flavonoid, induces autophagy in PC-12 cells by activating AMPK through the regulation of the AMPK-p70S6 kinase (p70S6K) signaling cascade, clearing α -synuclein^{A53T} and enhancing autophagy-related 7 (ATG7)-dependent autophagy [44].

In healthy individuals, dendritic cells (DCs) are either absent or found in low numbers in the central nervous system (CNS). In the early stages of PD development, peripheral DCs cross the blood-brain barrier (BBB) and react to α -synuclein aggregates, causing neuroinflammation. Ng et al. demonstrated that fibrillar α -synuclein impaired autophagy in monocyte-derived DCs (MoDCs). Treating cells with celastrol (0.25 μ M), isolated from *Tripterygium wilfordii* Hook F., induced autophagosome formation, decreased p62 expression, and increased the LC3-I/LC3II ratio. In addition, this treatment colocalized fibrillar α -synuclein with autophagy markers, indicating that celastrol contributes to the clearance of α -synuclein fibrils from MoDCs [45-47]. *Coptis chinensis* rhizome extract, widely used in traditional Chinese medicine, contains alkaloids including berberine, coptisine, and palmatine. Treating SH-SY5Y cells containing GFP-labeled α -synuclein^{A53T} using the extract (100 mg/mL) or its constituents (10 mM) significantly induced autophagy via the transcription factor EB (TFEB)-mediated pathway and reduced the aggregated protein by 70 percent [48]. GM1 ganglioside, a brain ganglioside that modulates neuroplasticity and releases neurotransmitters, ameliorated α -synuclein accumulation and reduced neurotoxicity by inducing α -synuclein clearance in 1-methyl-4-phenylpyridinium (MPP⁺)-induced SH-SY5Y cells. Treating PC-12 cells containing α -synuclein^{A53T} with 40 mg/mL of GM1 ganglioside induced autophagy by regulating the levels of autophagy-related 13 (ATG13), phosphorylated (p)-ATG13 (Ser355), ULK1, and p-ULK1 (Ser555). In a 1-

methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP; 30 mg/kg)-induced mouse model of PD, treatment with 50 mg/kg GM1 ganglioside improved motor and behavioral deficits [49].

Recent research indicates that lysosomal clustering around the microtubule-organizing center (MTOC) is vital for regulating autophagic flux. This arrangement increases the fusion of lysosomes and autophagosomes while inhibiting mTORC1, thus increasing autophagy [50,51]. PD patients form α -synuclein aggregates around the MTOC, indicating that MTOC-centered autophagy may contribute to clearing them [52]. Based on this hypothesis, Date et al. examined multiple compounds that promote lysosomal clustering near MTOCs, potentially reducing the distance between lysosomes, autophagosomes, and degradation substrates. Among teniposide, amsacrine, albendazole, etoposide, and mebendazole, 10 μ M albendazole enhanced lysosomal accumulation. Albendazole degraded α -synuclein aggregates via JNK-interacting protein 4 (JIP4)-mediated lysosomal positioning, and by phosphorylating the JIP4/mucolipin TRP cation channel 1 (MCOLN1/TRPML1)/ALG2 α -1,3/1,6-mannosyltransferase (ALG2) complex [53,54].

Histone deacetylases (HDACs) may regulate autophagy by modulating the acetylation levels of specific histone sites and autophagy-related ATG proteins. Acetylation of histone H3 at lysine 56 (H3K56ac) and histone H4 at lysine 16 (H4K16ac) is strongly associated with autophagy pathway regulation [55,56]. HDAC4 is abundant in brain tissue and reportedly colocalizes with α -synuclein. Several HDAC inhibitors, including trichostatin, butyrate, benzyl butyrate, and valproate, have been investigated for the treatment of PD [57,58]. Wang et al. found that Mc1568, a selective HDAC4 inhibitor, induced autophagy in a rotenone-induced SH-SY5Y cell model of PD. Treatment with 10 mM Mc1568 increased the LC3-I/II ratio and reduced aggregated protein levels by 50-fold [59].

In macroautophagy, sequestosome 1 (SQSTM1/p62) and lysosomal-associated membrane protein 1 (LAMP1) play crucial roles in protein degradation through autolysosome formation [61,62]. KW6002, also known as istradefylline, is an adenosine A2A receptor inhibitor that was tested for its autophagic efficiency in α -synuclein clearance. α -synuclein^{A53T} protofibrils were injected into male C57BL/6 mice and transfected mouse hippocampal HT-22 cells. KW6002 regulated the expressions of SQSTM1 and LAMP1, influencing autolysosome formation in the prefrontal cortex neurons of treated mice. In HT-22 cells, KW6002 modulated the expression of autophagy-related proteins LC3-I/II, SQSTM1, LAMP2A, and heat shock cognate protein 70 (HSC70). Interestingly, KW6002 also induced molecular CMA by

upregulating the heat shock protein 70 (HSP70) family, which recognizes the α -synuclein KFERQ motif and transports it to the autolysosome for degradation, clearing the aggregated protein by $20 \pm 4.5\%$ [63]. Recently, treatment of PD mice (α -synuclein^{A53T}) and PC-12 cells with 40 mg/kg and 30 mM, respectively, of the autophagy inducer harmol was reported to activate the AMPK/mTOR/TFEB pathway to induce autophagosome-lysosome biogenesis. Treating PC-12 cells with harmol also induced ULK1 phosphorylation at Ser757, which is involved in autophagosome formation [64].

Activating the autophagy initiator downstream protein is a promising strategy for inducing autophagy. Ferruginol is a natural phenol isolated from the needles of the redwood *Sequoia sempervirens* and the immature cones of *Chamaecyparis lawsoniana*. Treating α -synuclein^{A53T} transgenic C57BL/6 mice with 50-100 mg/kg of ferruginol induced lysosome-mediated autophagy and reduced protein aggregate levels [65]. *Caenorhabditis elegans* is a popular model of PD with several biological advantages, including the ease of visualizing its eight well-characterized dopaminergic neurons and the ability to monitor behaviors associated with their function [66]. Treatment of a *C. elegans* PD model (NL5901 [pkIs2386(Punc-54:: α -synuclein::YFP + unc-119)]) with HSEA-P1 and -P2-palmitic acids isolated from the sea cucumber *Holothuria scabra* Jaeger-reduced α -synuclein aggregation in muscle cells. The autophagy-related genes *bec-1* (BECN1), *lgg-1* (LC3), and *atg-7* (ATG7) were necessary for this degradation, and their expression was regulated by these compounds [67].

Purinergic receptor P2RX4, a member of the P2X receptor family, acts as a cation-permeable ion channel activated by the binding of extracellular ATP. Typically found on the plasma membrane, P2RX4 can be internalized into late endosomes, lysosomes, or lysosome-related organelles. Under normal conditions, P2RX4 is stable within lysosomes; however, its activation induces lysosomal Ca^{2+} release, promoting membrane fusion with autophagosomes. These insights suggest that P2RX4 could be a therapeutic target for modulating the ALP [68-71]. At 25 mM, piperine, an alkaloid isolated from *Piper longum* L., induced ALP activation and degraded α -synuclein aggregates in human neuroblastoma SK-N-SH cells. In a PD mouse model, treatment with 100 mg/kg piperine induced autophagy by activating P2RX4 [72].

Immune system and α -synuclein clearance

Immune cell activation in response to α -synuclein aggregation causes neuroinflammation, contributing to PD progression. α -synuclein aggregates stimulate brain-resident glial cells, including microglia and astroglia, inducing neuroinflammation [74-76]. Uncontrolled

microglial activation leads to higher expression of major histocompatibility complex antigens and the secretion of pro- and anti-inflammatory cytokines. As PD progresses, microglial activation causes the release of inflammatory cytokines such as tumor necrosis factor alpha (TNF- α), interleukins 1 beta (IL1 β) and 6 (IL6), and interferon-gamma (IFN- γ) in the substantia nigra [77,78]. Evidence from both *in vitro* (SH-SY5Y cells) and clinical experiments suggests that α -synuclein aggregation also induces neuroinflammation through the interleukin 6 cytokine family signal transducer-antisense (IL6ST-AS)/signal transducer and activator of transcription 3 (STAT3)/hypoxia-inducible factor 1 subunit alpha (HIF1- α) axis [79]. Abnormal α -synuclein aggregation also activates the NLR family pyrin domain containing 3 (NLRP3) inflammasome in microglia via TLRs. This leads to nuclear factor kappa B (NF- κ B) translocation, pro-inflammatory cytokine release, mitochondrial dysfunction, and subsequent damage to dopaminergic neurons [80].

Besides initiating the inflammatory response, microglial cells play a direct role in clearing α -synuclein aggregates. Microglia are brain-resident glial cells that regulate various functions in CNS, including the maintenance of neuronal networks and injury repair [81]. Microglial autophagy is also essential for suppressing neuroinflammation in the CNS. Extracellular α -synuclein can inhibit microglial autophagy by dysregulating the TLR4-dependent p38 mitogen-activated protein kinase (MAPK) and protein kinase B (AKT)-mTOR signaling cascade [82]. Microglia clear extracellular α -synuclein via phagocytosis or autophagy by recognizing α -synuclein aggregates with TLRs, Fc-gamma receptors (Fc γ Rs), and scavenger receptors [83]. Once α -synuclein is recognized, intracellular signaling pathways become activated and lead to phagosome formation, which fuse with lysosomes to form phagolysosomes. Several lysosomal enzymes subsequently degrade the α -synuclein aggregates [84,85]. Choi et al. demonstrated the importance of TLR4 in inducing transcriptional upregulation of p62/SQSTM1 in both primary microglial cells and mouse models. p62 induction initiates autophagy, inducing microglia to clear α -synuclein aggregates via TLR4/NF- κ B/p62-mediated synucleinopathy [86,87]. In addition, Rocha et al. showed that knocking out microglial NF- κ B/IKK $_2$ inhibited p62/SQSTM1-dependent autophagy and enhanced α -synuclein aggregation in a rotenone mouse model [88], suggesting that microglia are important in clearing accumulated α -synuclein.

Alongside microglia, astrocytes also contribute to neuroinflammation and the clearance of extracellular α -synuclein. α -synuclein triggers astrocyte activation, exacerbating neuroinflammation and neurodegeneration [89,90]. α -synuclein preformed fibrils (PFFs) activate

human midbrain astrocytes, causing inflammation, impaired phagocytosis, and neurotoxic effects via receptor-interacting serine/threonine kinase 1 (RIPK1) and 3 (RIPK3)-dependent NF- κ B signaling. This observation revealed a novel role for α -synuclein in promoting neurotoxic astrocyte activation and cell death-independent functions of RIPKs in neuroinflammation and parkinsonian neurodegeneration [91]. TLR2 activation in astrocytes exacerbates α -synuclein aggregation and propagation by impairing the ALP, contributing to neuronal cell death [92]. Despite their potentially detrimental effects, astroglia also contribute to neuroprotection by facilitating the clearance of α -synuclein aggregates via mechanisms such as phagocytosis, lysosomal degradation, and extracellular proteolysis [93,94]. Tsunemi et al. demonstrated that astrocytes internalize α -synuclein, with a higher rate of lysosomal degradation than in neurons. They also found that coculturing neuronal cells with astrocytes reduced α -synuclein accumulation within neurons, limiting transfer between them [95]. α -synuclein was degraded through the ALP in astrocytes, and this clearance was enhanced by ginkgolide K [96]. Recently, Yang et al. demonstrated that astrocytes enhance neuronal autophagic clearance of α -synuclein through paracrine signaling, indicated by the colocalization of p- α -synuclein (Ser129) with autolysosomes. They also showed that transplanting ventral midbrain astrocytes into a PD mouse model reduced α -synuclein pathology and neuron degeneration [97]. These findings suggest the importance of microglia and astrocytes in clearing α -synuclein aggregates.

Immunotherapy

Active immunotherapy

Active immunity is the process by which a specific antigen stimulates the immune system to produce antibodies and immune cells [98]. It can occur naturally after infection or artificially via immunization. In PD, active immunization aims to train the immune system to recognize and target pathological forms of α -synuclein. By generating a sustained immune response, active immunization holds promise for reducing toxic α -synuclein accumulation, slowing PD progression, and offering a long-term therapeutic benefit [99] (Table 2). Studies have investigated active immunization against α -synuclein using transgenic mouse models to assess vaccine effectiveness and safety. An α -synuclein vaccine was shown to notably reduce α -synuclein pathology, characterized by reduced accumulation of α -synuclein aggregates and decreased neuronal degeneration [100-102]. AFFITOPE (AFF) vaccines are peptide-based and designed to stimulate the production of antibodies against

α -synuclein [103,104]. Lemos et al. demonstrated that either alone or in combination, AFF PD03 and Anle138b improved gait deficits and preserved nigral dopaminergic neurons in a proteolipid protein (*Plp*)- α -synuclein mouse model; these effects were accompanied by reduced α -synuclein oligomers, decreased microglial activation, and lower immunoglobulin G (IgG) binding, ultimately slowing neurodegeneration. Clearance is believed to be mediated by microglia, resulting in increased expression of anti-inflammatory cytokines [105].

Based on promising preclinical findings, two Phase I clinical trials have examined the AFF PD01A and PD03A vaccines, assessing safety and tolerability in patients with PD and multiple system atrophy (MSA). The first, a randomized parallel-group Phase I trial, enrolled 32 patients with early-stage PD to evaluate vaccine effects. PD01A and PD03A were both safe and well-tolerated, and PD01A triggered a rapid and long-lasting antibody response [106]. The other trial assessed the safety, tolerability, and immunological activity of PD03A in 36 patients with early-stage PD randomized to receive 15 μ g or 75 μ g of PD03A or placebo over 52 weeks. Of these 36 patients, 35 completed all five immunizations. While all patients experienced adverse events, most were mild and unrelated to the treatment, with injection site reactions being the most common. A strong IgG antibody response was observed, peaking at week 12 and remaining significantly elevated in both active groups compared to the placebo group from weeks 8 to 52. The favorable safety profile and immune response support the further development of PD03A as an active immunotherapy for PD [107].

UB-312 is a synthetic peptide-based vaccine formulated to overcome immune tolerance and generate a humoral antibody response against oligomeric and fibrillary forms of α -synuclein. One study assessed the effect of active immunization against α -synuclein using the UB-312 vaccine in the thymus cell antigen 1 theta (*Thy1*)- α -synuclein/15 mouse model of PD. Young transgenic and WT mice underwent a six-week immunization regimen followed by a nine-week observation period. UB-312 immunization specifically targeted and attenuated oligomeric α -synuclein levels within the cerebral environment by 28%-50%. However, no statistically significant reduction in monomeric α -synuclein levels was observed, suggesting that UB-312 does not promote the oligomer disassembly into monomers but instead promotes their clearance from the CNS. However, this study did not directly discuss the clearance of oligomers through autophagy [108]. A randomized placebo-controlled Phase I clinical trial evaluated the safety, tolerability, and immunogenicity of UB-312 in 50 healthy participants who received three intramuscular injections at varying doses, or placebo. UB-

312 was found to be generally safe, well-tolerated, and immunogenic, with a strong antibody response, including the ability to cross the BBB [109]. However, challenges remain, such as immune tolerance to self-proteins and effective antibody delivery across the BBB, which must be addressed for effective therapeutic outcomes [110].

Passive immunotherapy

Passive immunity refers to the production of IgG antibodies to protect against infections. It can be natural, lasting weeks to months, or acquired through the injection of concentrated immune serum. Passive immunization for NDDs involves the administration of externally engineered antibodies targeting specific α -synuclein domains, mimicking the action of endogenous IgG. In PD treatment, these antibodies bind to extracellular α -synuclein to prevent its spread [98,111,112] (Table 2).

Table 2. Preclinical and Clinical studies of active and passive immunotherapy for α -synuclein clearance.

Type	Peptide	Study model	Dose	Duration of treatment	Target site	Therapeutic effects	Refs.
Active	PD03 and Anle138b	PLP α -Syn mice	PD03 = subcutaneous injection (200 μ l each), Anle138b = food pellets containing 0.6 g compound/kg of food	5 months	C-terminus of α -Syn	Reduced α -Syn oligomer level and microglial activation	[105]
	UB-312	Thy1SNCA/15 mice	40 μ g per injection	15 months	C-terminus of α -Syn	The level of α -Syn was reduced	[108]
Passive	Prasinezumab (PRX002)	Healthy individuals	0.3, 1, 3, 10, or 30 mg/kg, intravenous infusion	a single dose	C-terminus of α -Syn	Reduced free serum α -Syn	[114]
	Phase I						
	Phase II	PD patients	1,500 or 4,500 mg	3 intravenous infusions once every 28 days	C-terminus of α -Syn	The result is expected to be published in 2026	[115]
	Phase II	PD patients	1,500 or 4,500 mg	intravenous infusions once every 4 weeks for 52 weeks	C-terminus of α -Syn	Much slower worsening of motor symptoms compared to the control group	[116]
	BIIB054 (Cinpanemab)	α -synA53T (M83), BAC- α -synWT, BAC- α -synA53T mice	30 mg/kg	intravenous infusions, weekly for 180 days	N-terminus (residues 1–10) of α -synuclein	Decreased α -Syn spreading and improved motor performance	[117]
	Phase I	Healthy individuals & PD patients (NCT02459886)	15 or 45 mg/kg	Single IV dose, 16 weeks	N-terminus (residues 1–10) of α -synuclein	Showed selectivity for the aggregates, found safe and crossed BBB	[118]
	Phase II	Phase II (SPARK): Mild to moderate PD (NCT03318523)	250, 1,250, or 3,500 mg	Every 4 weeks for two years	N-terminus (residues 1–10) of α -synuclein	Discontinued (did not meet primary or secondary endpoints)	[119]
	MEDI1341	Sprague Dawley Rats	3 mg/kg to 100 mg/kg	Intravenous, for 13 weeks	C-terminus of α -Syn	Reduced CSF fluid α -Syn levels $81.5 \pm 4.6\%$ at the top dose of 100 mg/kg	[120]
		Cynomolgus monkeys	25, 75, and 150 mg/kg			Decreased CSF fluid α -Syn levels $88 \pm 1.8\%$ at the top dose of 150 mg/kg	
		Lentivirus- α -synuclein-expressed mice	20 mg/kg	Intraperitoneal (IP), for 13 weeks		Decreased α -Syn propagation along axons	

ABBV-0805 (BAN0805)	Thy1-A30P and α -synA53T mice	0.1, 1, 10, and 20 mg/kg	Intravenous, Intraperitoneal, 2-4 months	C-terminus of oligomers & protofibrils	Decreased soluble and insoluble α -Syn Increased mean survival of A30P mice	[110, 121]
Lu AF82422	cynomolgus monkey	100–600 mg/kg	Intravenous, 56 days	C-terminus	Reduced α -Syn in plasma and CSF	[122]
DR5-12D	NKA α 1+/+ and NKA α 1+/- mice	30 mg/kg	Intraperitoneal for 83 days	-	DR5-12D activates NKA α 1-dependent autophagy by enhancing signaling through the AMPK/mTOR/ULK1 pathway and clears aggregated protein	[123]

Abbreviations: NKA α : Na⁺/K⁺-ATPase- alpha subunit

The first Phase 1 clinical trial examining the anti- α -synuclein antibody PRX002 involved 40 healthy adults who received a single intravenous infusion of 0.3, 1, 3, 10, or 30 mg/kg. PRX002 effectively lowered free serum α -synuclein levels, suggesting clearance from the peripheral circulation. This reduction occurred quickly, within an hour of administration, and was dose-dependent. These findings suggest that clearance may be mediated by microglial activity and neuronal autophagy. PRX002 was also found to be safe and well-tolerated [113]. Later, the PASADENA trial, a two-year Phase II clinical trial by Roche, evaluated the efficacy of prasinezumab (1,500 or 4,500 mg) in patients with early PD. Its primary aim was to assess motor function improvement using the Movement Disorder Society-Unified Parkinson's Disease Rating Scale. However, the latest 52-week report indicated no significant difference between the treatment and placebo groups. The trial is expected to conclude in 2026 [114, 115]. Another Roche clinical trial, PADOVA Phase IIB, involves 575 patients with more advanced PD symptoms than those in the PASADENA trial. These patients are on stable dopamine agonists and receive monthly injections of prasinezumab or a placebo for 18 months. The trial's primary outcome is motor progression, with secondary outcomes of motor function and infusion reaction, the most common side effect. Prasinezumab treatment slowed motor progression in PD over 4 years, with delayed- and early-start groups showing 51%-65% slower decline in MDS-UPDRS Part III (OFF), 94%-118% slower decline in Part III (ON), and 40%-48% slower decline in Part II scores compared to an external comparator. These findings suggest a sustained long-term benefit, but must be confirmed in further studies [116].

Biogen's monoclonal antibody (mAb), BIIB054, is a recombinant human IgG1 antibody produced by the memory B cells of older adults without neurological disorders. It binds to the N-terminus (residues 1–10) of α -synuclein. In a preclinical study, 30 mg/kg BIIB054 was administered to transgenic α -synuclein^{A53T} (M83), transgenic bacterial artificial chromosome (BAC)- α -

synuclein^{WT}, and transgenic BAC- α -synuclein^{A53T} mice via intraperitoneal injection. This treatment decreased α -synuclein spreading by 30%, dopamine transporter (DAT) density loss in dopaminergic terminals by 20%, and improved behavioral performance in the wire-hanging test by 50% [117]. Due to the promising effects, a randomized, double-blind, placebo-controlled clinical trial (NCT02459886) evaluated the safety and pharmacokinetics of BIIB054 (cinpanemab) in healthy individuals and patients with mild to moderate PD. Participants received a single dose (15 or 45 mg/kg) of BIIB054 or placebo and were assessed through clinical, neuroimaging, electrocardiography (ECG), and laboratory measures for 16 weeks. BIIB054 exhibited a favorable safety profile, crossed the BBB, and was more selective for α -synuclein aggregates; however, it bound soluble α -synuclein at high doses. Biogen later initiated the SPARK Phase II clinical trial (NCT03318523) to further investigate the safety and pharmacokinetics of BIIB054 over two years; however, this trial was discontinued because neither the principal nor ancillary objectives were achieved [118,119].

MEDI1341 is a highly specific, high-affinity mAb (epitope: AA 103-129) that binds monomeric and aggregated α -synuclein, effectively inhibiting its spread. Preclinical studies have confirmed its ability to rapidly penetrate the brain and significantly reduce free α -synuclein levels in cerebrospinal fluid (CSF) and brain interstitial fluid (ISF). MEDI1341 inhibited α -synuclein PFF in SH-SY5Y cell models, while intravenous administration of 100 mg/kg in rats reduced α -synuclein levels by a peak of 81.5% in CSF α -synuclein and 75% in brain ISF. In cynomolgus monkeys, CNS penetration reached 0.2% of serum levels, with up to 88% reduction in CSF α -synuclein. MEDI1341 (TAK-341) is under Phase I clinical trials for safety, pharmacokinetics, and CNS target engagement. These trials involved healthy volunteers and patients with PD and were later modified to include eye health assessments. In November 2022, Takeda took over development, launching a Phase II

clinical trial involving 138 patients with MSA, focusing on changes in the Unified MSA Rating Scale and other clinical measures. However, no results have been disclosed at this time [110,120].

AN0805/ABBV-0805 (BAN0805) is a humanized mAb that specifically targets the C-terminus of α -synuclein oligomers and protofibrils. Two different preclinical studies have examined its effects in two mouse models of PD, one expressing human Thy1-A30P and the human α -synuclein^{A53T}. The mice received ABBV-0805 at doses from 0.1 to 20 mg/kg over 2-4 months, with treatment effects evaluated through α -synuclein levels, motor function, and survival. ABBV-0805 reduced α -synuclein fibrils, improved motor function, and extended survival, demonstrating its protective effects against α -synuclein toxicity and potential as a PD therapy [110,121].

Lu AF82422 is a human IgG1 mAb that targets all major forms of α -synuclein, including monomeric, oligomeric, and truncated forms. In a single-dose pharmacokinetics (PK) study in cynomolgus monkeys, LuAF82422 demonstrated typical IgG1 mAb properties, including linear PK and a plasma half-life of 10-15 days. Only 0.1%-0.2% of the antibody reached the CSF. In a repeat-dose study, antibody exposure in plasma and CSF increased over time. α -synuclein levels in plasma and CSF showed dose-dependent reductions, but with high variability, possibly due to blood contamination or hemolysis. A significant decrease in free α -synuclein in CSF was observed at doses above 100-600 mg/kg, indicating target engagement. However, the variability in CSF data and the timing of peak drug concentrations (64-88 hours) suggested that higher doses or longer observation periods may be needed for optimal engagement. Modeling estimates suggest that doses of 15-60 mg/kg would effectively bind 85%-95% of oligomeric α -synuclein in CSF, with a safety factor of 10-40 [122]. None of the discussed trials provide clear evidence of these antibodies' ability to eliminate α -synuclein aggregation through autophagic pathways. There is also a noticeable gap in studies investigating their efficacy against mutated forms of α -synuclein. Therefore, further research is warranted to explore these aspects comprehensively.

The broader implications of immunotherapy for clearing α -synuclein aggregates remain under-researched. Cao et al. reported the development and testing of an antibody (DR5-12D) targeting the Na⁺/K⁺-ATPase (NKA) α 1 subunit (ATP1A1), which activates NKA-dependent autophagy [123]. NKA is a transmembrane protein that maintains the ion gradient in CNS cells. Evidence suggests that NKA deprivation can exacerbate neurodegeneration and cause PD [124-126]. NKA has also been reported to modulate autophagy [127,128]. The

DR5-12D mAb works by targeting the DR region (⁸⁹⁷DVEDSYGQWWTYEQR⁹¹¹) of ATP1A1, stabilizing and activating NKA. This enhances autophagy via the AMPK/mTOR/ULK1 pathway, promoting the degradation of α -synuclein aggregates. DR5-12D improved learning, memory, and motor function in a rodent model of PD induced by α -synuclein PFFs [123].

Peptide-based therapy for α -synuclein clearance

Small molecules dominate the drug market due to their affordability, bioavailability, and potential for oral administration. However, structural constraints render them inefficient against protein-protein interactions (PPIs). Antibodies and other larger biologics can target external PPIs, but not those that occur intracellularly. Through sophisticated peptide synthesis and purification techniques, short peptides provide a promising compromise; they exhibit high specificity to block PPIs, decreased toxicity, minimal risk of immune response, and economical manufacture [129-131]. In PD studies, peptides have mainly been designed to inhibit α -synuclein aggregation rather than clear it from cells (Table 3). To inhibit α -synuclein aggregation and reduce its toxicity, its N-terminal lipid binding domain was targeted using aS₁₋₂₅ (MDVFMKGLSKAKEGVVAAAEEKTKQG) [132], Tat- β syn-degron (YGRKKRRQRRRTKSGVYLVGR RRG) [133], and NTR-TP-L and -D (GVLYVGS-Aib) [134]. The NAC domain responsible for amyloid aggregation has been targeted using S37 (GAVVWGVTAVKKKKK), S61 (GAVVWGVTAVGR KRRRQRRRPQ), S62 (GAVVWGVTAVKKGR KKR RQRRRPQ), and S71 (YGRKKRRQRRRAVVT[nme-Gly]VTAVAE) [135]. Recently, Nim et al. targeted its C terminus using PDpep1.3 (DEEIERQLKALG) [136]. K84s (FLVWGCLRGSAIGECVVHGGPPSRH), K102s (FLKRWARSTRWGTASCGGS) [137], and p216 (WFQNRMRMKWKK) [138] directly inhibited oligomers, while PSM α 3 (MEFVAKLFKFFKDLLGKFLGNN) and LL-37 (LLGDFFRKSKEKIGKEFKRIVQRI KDFLRN LVPRTES) [139] reduced type B oligomer fibrils.

Angiotensin-(1-7) has exhibited neuroprotective effects in PD [140-142]. Continuous infusion (1.1 nmol/0.25 μ L/hour) into the substantia nigra decreased α -synuclein aggregation and parkinsonian behaviors in a rotenone-induced rat model. At 100 nM, angiotensin-(1-7) also reversed autophagic dysfunction, reducing α -synuclein accumulation. Western blots revealed increased p62 levels and decreased LC3-II levels in neurons treated with rotenone, suggesting defective autophagy. By regulating LC3-II and p62 levels and dramatically increasing autophagic activity, co-treatment with angiotensin-(1-7) may help to restore autophagic function [143]. Cathepsin L (CTSL) is a downstream lysosomal

cysteine protease essential for protein degradation [144]. Its overexpression in human embryonic kidney HEK293F cells expressing enhanced GFP (eGFP)-tagged α -

synuclein induced autophagy and promoted clearance of α -synuclein^{WT} [145].

Table 3. Peptide-based autophagy inducers.

Peptide	Study model	Dose	Duration of treatment	Therapeutic effects	Refs.
<i>In vitro study</i>					
Angiotensin-1-7	Rat midbrain dopaminergic neurons	100 nM	24 hours	Blocking the MAS receptor increased the LC3-II/LC3-I ratio, reduced α -synuclein aggregation in the substantia nigra, improved behavior, induced autophagy, and cleared the aggregated protein.	[143]
CTSL	HEK293F/ α -synuclein-EGFP cells	Overexpression	-	Induced autophagy via ATG5/LC-3, facilitating the clearance of α -synuclein fibrils from the cell.	[145]
ATACC peptide	HEK293T cells	0–100 μ M	24 hours	Autolysosome formation, enhanced LC3-II/LC3-I ratio, and degraded α -synuclein aggregates.	[146]
KYP-2407	SH-SY5Y cells	10 μ M	48 hours	Increased autophagy, normalized CAPN1 and CAPN2 levels, and cleared α -synuclein.	[152]
<i>In vivo study</i>					
Angiotensin-1-7	Sprague–Dawley rats	1.1 nmol/0.25 μ L/hour, Continuous supranigral infusion	4 weeks	Blocking the MAS receptor increased the LC3-II/LC3-I ratio, reduced α -synuclein aggregation in the substantia nigra, improved behavior, induced autophagy, and cleared the aggregated protein.	[143]
ATACC peptide	C57BL/6 mice	50 mg/kg/b.w./day, Intravenous injection	5 days	Autolysosome formation, enhanced LC3-II/LC3-I ratio, and degraded α -synuclein aggregates.	[146]

Abbreviations: α -syn: Alpha synuclein; LC3: Microtubule-associated protein 1A/1B-light chain 3ATG: Autophagy-related protein; b.w: Body weight.

One method for reducing α -synuclein aggregation is targeted protein degradation (TPD). While many proteins are degraded via the ALP, few TPD tools exploit this route. Tong et al. developed four autophagosome-anchoring chimeras (ATACCs). ATACCs promote selective autophagy by employing a bifunctional peptide containing an LC3-interacting region (LIR) to anchor proteins of interest (POIs)-such as α -synuclein-to LC3B on autophagosomes. In both *in vivo* and *in vitro* models, the peptide successfully degraded the aggregated protein via autophagy. An α -synuclein overexpressing HEK293T cell line was used to assess the degrading effects on α -synuclein. α -synuclein was knocked down by all four ATACC peptides in a dose-dependent manner. Peptide P1 exhibited the greatest effectiveness, with a half-maximal degradation concentration (DC₅₀) of $46.8 \pm 3.7 \mu$ M [146]. Prolyl endopeptidase (PREP) is a serine protease that directly interacts with α -synuclein to promote dimerization and aggregation, independent of its enzymatic activity. This interaction is associated with the formation of α -synuclein aggregates observed in patients with PD and dementia with Lewy bodies [147]. PREP inhibitors have been reported to induce autophagy and

promote α -synuclein clearance both *in vivo* and *in vitro* [148-151]. Rostami et al. investigated the effect of KYP-2047 on intracellular α -synuclein PFFs in SH-SY5Y cells and human astrocytes. Confirmed by immunostaining and western blots, 10 mM KYP-2047 decreased intracellular α -synuclein deposits in SH-SY5Y cells but not in astrocytes, which lack PREP activity. In addition, KYP-2047 reduced the levels of autophagy-related proteins p62 and light chain 3 beta 2 (LC3B2) in SH-SY5Y cells, indicating increased autophagy. Moreover, the treatment normalized calpain 1 (CAPN1) and 2 (CAPN2) levels, counteracting their increase due to α -synuclein accumulation [152].

Peptide-based therapies offer a promising alternative for inhibiting α -synuclein aggregation and promoting its clearance in PD. TPD- and autophagy-enhancing peptides represent novel strategies to facilitate α -synuclein clearance. Ongoing research continues to explore peptide-based approaches as potential disease-modifying treatments for PD.

Other autophagy inducers for α -synuclein clearance

Traditional Chinese medicines reportedly act as autophagy inducers to reduce the risk of NDD [153,154]. The ethanol extract of *Artemisia argyi* H. Lév. & Vaniot (60-80 μ g/mL) promoted the clearance of α -synuclein aggregates in MPP⁺-induced SH-SY5Y cells in a dose-dependent manner. It also increased levels of TRPML1-a nonselective cation channel located in lysosomal membranes-and enhanced lysosomal activity. Activation of TRPML1 can trigger autophagy, which is significantly increased by *A. argyi* extract [155-157]. Another popularly used traditional Chinese medicine, *C. chinensis* rhizome extract, also triggered autophagy via the TFEB-mediated pathway in SH-SY5Y cells expressing GFP-labeled α -synuclein^{A53T} [158].

Nanoparticles can successfully cross the BBB and are used in PD to deliver active compounds to dopaminergic neuron-rich areas. Various nanoparticles are reported to exhibit different mechanisms for combating PD [150]. Gold nanoclusters were found to have anti-fibrillation properties against α -synuclein aggregation, while melatonin-loaded polymeric nanoparticles reduced α -synuclein fibrillation *in vitro* [159,160]. Gold nanoparticles, such as CNM-Au8, are reported to protect dopaminergic neurons against oxidative stress in MPP⁺- and 6-hydroxydopamine (6-OHDA)-induced lesions [161]. Recently, significant progress has been made in inorganic (metal, quantum dot, and cerium oxide) and organic (polymeric, solid, and liposome) nanoparticles [162]. Quercetin-modified Cu_{2-x}Se (CSPQ), an enzyme-like nanoparticle, exhibits effective ROS scavenging activity [163] and successfully clears α -synuclein aggregates. In α -synuclein PFF-treated primary neurons isolated from C57BL/6 mice, 4 μ M CSPQ nanoparticles significantly reduced α -synuclein aggregation [164].

New technology for targeted protein degradation (TPD) in PD

Traditional therapies primarily aim to manage symptoms without addressing the underlying cause of protein accumulation. In recent years, TPD has emerged as a promising therapeutic strategy that directly eliminates disease-driving proteins by co-opting a cell's degradation machinery [165]. Unlike conventional inhibitors that block protein function, TPD approaches-including PROTACs (proteolysis-targeting chimeras), molecular glues, and autophagy-based degraders-aim to selectively and efficiently degrade toxic membrane proteins and aggregated proteins such as α -synuclein. By promoting the clearance of pathogenic proteins, TPD offers the potential for disease modification and neuroprotection in PD, marking a significant advancement in the development of next-generation therapeutics [166].

Autophagosome-tethering compound (ATTEC) technology is an emerging method for degrading POIs. Unlike conventional therapies, ATTECs harness autophagy directly to eliminate specific disease-causing proteins. By linking POIs to the autophagy machinery, ATTECs can potentially target a wide range of proteins, even those previously considered undruggable [167,168]. However, challenges remain in designing target-specific molecules. One study combined an ATTEC with aptamers, highly specific and affinity-purified DNA or RNA oligonucleotides [169], to create targeted protein degraders. Using α -synuclein as a model pathological protein, a biorthogonal click reaction was used to conjugate an α -synuclein-targeting aptamer with the LC3-binding compound 5,7-dihydroxy-4-phenylcoumarin (DP). The resulting aptamer-DP conjugate successfully degraded α -synuclein aggregates through autophagic pathways. This approach demonstrates the potential of aptamer-based ATTECs for designing versatile and specific degraders for various proteins [170].

Recently, an autophagy-targeting chimera (AUTOTAC) has been developed, enabling TPD therapeutics employing p62-mediated autophagy. This compound utilizes a chimeric molecule consisting of a target-binding ligand (TBL) connected to an autophagy-targeting ligand (ATL) that interacts with the ZZ domain of p62 [171]. Anle138b is a chemical compound used as an aggregation inhibitor for α -synuclein and is currently being examined in a Phase 1b clinical trial involving patients with PD (NCT04685265) [172,173]. AUTOTAC uses anle138b as a TBL component to specifically target α -synuclein, while its ATL component binds to p62, promoting its self-oligomerization within its complex. This facilitates the recruitment of aggregates to LC3⁺ autophagic membranes, leading to lysosomal degradation at a concentration of 1 mM in PD model cells (HeLa, COS7, and HEK293T). The degradation process selectively targets detergent-insoluble α -synuclein aggregates without affecting functional, monomeric α -synuclein in SH-SY5Y cells. It also reduced cell-to-cell transmission of α -synuclein aggregates in cultured cells. In PD model mice, oral administration of 10 mg/kg of ATC161 demonstrated therapeutic efficacy by degrading α -synuclein aggregates in the brain, improving glial immune responses, and slowing the progression of motor deficits [174].

Limitations and future perspectives

Proteins targeted by CMA typically contain a recognition motif that is identified by the HSC70 chaperone, which guides them to the lysosomal membrane. α -synuclein possesses such a motif and is normally degraded via CMA. Disease-associated mutant forms of α -synuclein,

such as α -synuclein^{A53T}, are still capable of binding to the LAMP2A receptor but exhibit severely impaired translocation into the lysosomal lumen. As a result, these mutant proteins accumulate at the lysosomal surface and can interfere with normal CMA functioning, contributing to protein aggregation and cellular stress in neurodegenerative conditions [175,176]. This results in the activation of macroautophagy as an alternative degradation pathway. Unfortunately, it has been demonstrated that α -synuclein^{A53T} and α -synuclein^{E46K} aggregates are not degraded via autophagy, even when a lysosomal cysteine protease, CTSL, is overexpressed [145,177]. Therefore, thoroughly investigating autophagy activation for mutant α -synuclein degradation is crucial. Additionally, few studies have examined the clearance of other mutant α -synuclein aggregates. For instance, no studies have explored the clearance of the aggregated form of α -synuclein^{A30P}, another aggregate-prone mutant, with reports only published on its monomer form [178,179].

Studying the long-term effects of hyperactive autophagy on cell survival is necessary, as excessive autophagy has been associated with various mechanisms of cell death. This has led to the use of the term “autophagic cell death” to describe cell death occurring alongside autophagy [180,181]. Overactive autophagy can be pathogenic [182], and chronic activation of the process can lead to neurodegeneration and neuronal death. Zhang et al. reported that chronic corticosterone (CORT) treatment of C57BL/6J mice hyperactivated autophagy by increasing autophagy-related 5 (*ATG5*) expression. This led to excess lysosomal degradation of brain-derived neurotrophic factor (BDNF) in dopaminergic neurons and subsequently impaired neurogenesis [183]. Therefore, research is needed to determine the point at which autophagy regulation is optimized to maintain neuronal function.

Inhibiting α -synuclein aggregation is the most common approach to preventing PD progression. However, these aggregates are already present in the brains of patients by the time symptoms manifest. Aggregation inhibitors may prevent new aggregates forming but cannot remove those already present. Enhancing autophagic clearance can actively degrade pre-existing toxic aggregates, potentially reversing or mitigating disease progression. In addition, autophagic pathways can target a range of α -synuclein forms, including smaller toxic intermediates and larger aggregates. Associated treatments would be applicable even in advanced stages, targeting existing aggregates regardless of disease progression. Therefore, further *in vivo/in vitro* studies exploring how α -synuclein degradation can be enhanced are also important. While strategies to promote α -synuclein clearance via autophagy

activation are proposed as treatments, there is currently limited evidence regarding their clinical applicability and side effects.

Conclusion

Recent advances in understanding the clearance of α -synuclein aggregates via autophagy have highlighted its potential as a therapeutic strategy for NDDs such as PD and MSA. Autophagy-enhancing strategies hold strong therapeutic potential for the clearance of pathological α -synuclein in PD. Emerging approaches—such as ATACC, ATTEC, AUTOTAC, and CMA—offer novel and selective means to degrade aggregated or misfolded α -synuclein, including forms that are otherwise resistant to conventional clearance mechanisms. However, challenges remain, including the difficulty of identifying specific toxic α -synuclein species, the diverse nature of α -synuclein aggregation between patients, and the lack of reliable biomarkers for monitoring treatment efficacy. Despite growing interest in immunotherapeutic approaches for PD, there remains a significant gap in studies specifically targeting the elimination of mutant forms of α -synuclein. Likewise, the exploration of autophagy-based mechanisms for the selective degradation of these pathogenic variants is still in its infancy. Although most of the compounds discussed above have demonstrated adequate safety and tolerability profiles, only a limited number have shown the ability to effectively cross the BBB, a critical requirement for therapeutic efficacy in NDDs. Future research should focus on elucidating the molecular mechanisms regulating autophagy in α -synuclein-related pathologies and optimizing therapeutic interventions that leverage this pathway to enhance the clearance of pathological aggregates.

Author Contributions

Writing-original draft preparation with figures, Khoshnour Jannat and Lee-Sang Bong; writing-review and editing, Rengasamy Balakrishnan; Yon-Suk Kim; assistance with preparation of tables and data collection, Hong-Ryeol Jung and Mujeeb Ur Rahman; design and supervision of the study, Dong-Kug Choi. All authors have read and approved the final manuscript. Khoshnour Jannat and Lee Sang Bong contributed equally.

Conflicts of Interest

The authors declare no conflict of interest.

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