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# EXPERT OPINION

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## Targeting $\alpha$ -synuclein as a therapeutic strategy for Parkinson's disease

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**Introduction:**  $\alpha$ -Synuclein, a neuronal protein, plays a central role in the pathophysiology of Parkinson's disease (PD), the second most prevalent neurodegenerative disorder. Cases of PD have increased tremendously over the past decade necessitating the identification of new therapeutic targets to reduce patient morbidity and to improve PD patients' quality of life.

**Areas covered:** The purpose of this article is to provide an update on the role of  $\alpha$ -synuclein in fibrils formation and review its role as an effective immunotherapeutic target for PD. The rapidly expanding evidence for the contribution of  $\alpha$ -synuclein to the pathogenesis of PD led to the development of antibodies against the C terminus of  $\alpha$ -synuclein and other molecules involved in the inflammatory signaling pathways that were found to contribute significantly to initiation and progression of the disease.

**Expert opinion:** The readers will obtain new insights on the mechanisms by which  $\alpha$ -synuclein can trigger the development of PD and other related degenerative disorders along with the potential role of active and passive antibodies targeted against specific form of  $\alpha$ -synuclein aggregates to clear neurotoxicity, stop the propagation of the prion-like behavior of these oligomers and reverse neuronal degeneration associated with PD.

**Keywords:**  $\alpha$ -synuclein, immunotherapy, inflammation, Lewy bodies, neurodegenerative disorder, Parkinson's disease, synucleinopathies

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### 1. Introduction

Parkinson's disease (PD) is a chronic and progressive neurodegenerative disorder that develops chiefly from the loss of dopaminergic-producing cells of the substantia nigra (SN) and the accumulation of cytoplasmic inclusions of Lewy bodies (LBs) in surviving neurons. These accompanying neuropathological changes subsequently lead to the depletion of dopamine (DA) and disruption of neural activity in the basal ganglia. The filamentous inclusions are widely spread and appear frequently within the olfactory bulbs and locus coeruleus [1]. Their appearance, however, is not directly linked to the development of parkinsonian motor symptoms as clinical studies have shown that the loss of some DA-producing neurons in the SN may precede accumulation of LB pathology [2]. The etiology of PD is still poorly understood; nevertheless in the past decade, clinical and animal model studies have advanced our understanding of the pathogenesis of this disease, particularly with the identification of several causative factors, genetic and environmental, that may directly or indirectly trigger the symptoms of PD [3-5]. Recently, however, attention has specifically focused on the role of the 140 residue soluble neuronal proteins,  $\alpha$ -synuclein, in the initiation of the pathology associated with PD and other neurodegenerative diseases [6,7]. Overwhelming evidence has emerged showing

**Article highlights.**

- Overwhelming evidence is now available which supports the concept that misfolded  $\alpha$ -synuclein is involved in the initiation and pathology of Parkinson's disease (PD).
- Targeting this pathologic protein is now considered a novel therapeutic approach for the treatment of the neurodegenerative condition.
- The physiological role of  $\alpha$ -synuclein in the brain is not fully understood, even though it has been proposed that it has a role in neurotransmitter release and recent work suggests a role in membrane remodeling.
- Aggregation of this protein has been attributed to several potential factors including SNCA gene mutation, decreased rate of degradation or possible alteration of  $\alpha$ -synuclein, such as truncations, missense mutations.
- Inflammation in the brain, which can be triggered by dysregulated action of glial cells and astrocytes, which can be triggered by neurotoxic  $\alpha$ -synuclein, can contribute to pathogenesis of PD.
- Immunotherapy and novel anti-inflammatory approaches may contribute to new therapeutic methodologies.

This box summarizes key points contained in the article.

that overexpression and deposition of the misfolded and aggregated  $\alpha$ -synuclein proteins in the cytoplasm of selective populations of dopaminergic neurons [8], as well as their interaction with other proteins play a critical role in neuronal survival. Additional findings have bolstered that view and reshaped our understanding of the pathogenic mechanisms underlying PD, prompted the classification of PD as part of the synucleinopathy disease spectrum and implicated  $\alpha$ -synuclein as the prime contributor to PD development. Moreover, new comprehensively designed animal models [9,10] and *in vitro* experiments [11] have led to the identification of novel molecular pathways underlying  $\alpha$ -synuclein deposition and to the development of targeted therapies capable of harnessing the immune response and potentially alleviating the motor symptoms and improving patients' quality of life.

## 2. $\alpha$ -Synuclein: biological role and proposed mechanisms of aggregation

Over the past few years, research studies have provided a body of evidence showing that the misfolded  $\alpha$ -synuclein protein, the main constituent of the LBs, accumulate intraneuronally in the brains of patients with PD and other related disorders; however, the relationship between this protein and the initiation of symptomatic behaviors associated with PD remains poorly understood. Whereas the specific function of this protein has not been clearly established, overexpression of  $\alpha$ -synuclein has been shown to play a key role in synaptic plasticity and learning [12], in supplying synaptic vesicles in presynaptic terminals [13], in regulating vesicle trafficking and refilling [14] and in regulating DA biosynthesis by

reducing the action of tyrosine hydroxylase or altering its phosphorylation [15,16]. It is significantly upregulated in a distinct population of presynaptic terminals where it interacts with tubulin and affects its polymerization leading to the inhibition of microtubule formation in cultured cells [17]. The potential physiological role of  $\alpha$ -synuclein in regulating the dynamics of neural microtubule implies its involvement in intracellular transport, cellular metabolism and apoptosis. Furthermore, the fact that this protein exists in either a natively unfolded conformation or as an  $\alpha$ -helix in the presence of phospholipids [18] implies the involvement of a highly dynamic mechanism that regulates the function of  $\alpha$ -synuclein depending on the local cellular environment. The function of  $\alpha$ -synuclein is very closely related to its structure. Therefore, understanding the structure and the normal function of  $\alpha$ -synuclein is highly crucial to shed light on its involvement in neurodegenerative diseases and to gain a better insight into the particular role it plays in PD.

Structurally, the human  $\alpha$ -synuclein is a small acidic protein made of 140 amino acids (14.5 KDa) and is encoded by the SNCA gene. It contains three modular domains, including an amino-terminal lipid-binding  $\alpha$ -helix, a hydrophobic amyloid-binding domain that encodes the non-amyloid- $\beta$  component (NAC) of amyloid plaques and a carboxyl-terminal acidic tail. The amphipathic N-terminal region is dominated by four 11-residue repeats including the consensus sequence KTKEGV. This sequence has a structural  $\alpha$ -helix propensity similar to apolipoprotein-binding domains. The ability of this domain to adopt an  $\alpha$ -helical structure suggests that  $\alpha$ -synuclein is a membrane-bound protein. The central hydrophobic region, which includes the NAC region, is believed to be involved in protein aggregation by mediating the conformational changes of  $\alpha$ -synuclein from random coil to a  $\beta$ -sheet structure. Based on experimental mutation of the residues and various studies of synthetic peptides, the hydrophobic region (residues 71 – 82) has been found to be fundamental for the ability of  $\alpha$ -synuclein to oligomerize and form amyloid fibrils *in vitro*. In contrast, the highly acidic and proline-rich region has no distinct structural propensity. Although it comprises no significant secondary structure, it has a characteristic negative charge due to a high density of acidic amino acids. This C-domain contains three tyrosine residues that are significantly nitrated in cytosolic inclusions; however, the timing of nitration in relation to protein aggregate formation is not yet clear. A particular interest is a serine residue located at position 129. Cross-correlation of a Ser129 has been indicated to alter the hydrophobicity and charge distribution of the region and to promote  $\alpha$ -synuclein oligomerization. The structure of  $\alpha$ -synuclein provides evidence of an aggregation pathway [19]. Although  $\alpha$ -synuclein exists predominantly as a random coil, covalent modification suggests that phosphorylation at position Ser129, as well as hydrophobic interactions particularly at the NAC, allows for the polymerization of various  $\alpha$ -synuclein proteins into anti-parallel  $\beta$ -sheet conformation.

However, even though, there is a correlation between this modification and ease of synuclein aggregation, it is not strictly required for aggregation. Existing hydrogen bonding confers greater stability to the  $\beta$ -sheet, further amplifying the aggregation of potential  $\alpha$ -synuclein and permitting the formation of fibrils that drastically impact normal cellular processes. A number of studies have shown that phosphorylation of  $\alpha$ -synuclein at Ser129 is evident in PD and related synucleinopathies as demonstrated by the presence of a strong synergistic relationship between A $\beta$  accumulation and the phosphorylation of  $\alpha$ -synuclein at Ser129 in postmortem human brain tissue [20]. On the other hand, other studies suggest that  $\alpha$ -synuclein neurotoxicity in PD and related synucleinopathies may result from an imbalance between the damaging, oligomer-promoting effect of Ser129 phosphorylation and the neuroprotective action of tyrosine 125 phosphorylation that prevents cytotoxic oligomer accumulation during fibril formation [21,22].

### 3. $\alpha$ -Synuclein gene mutation: role in PD

The plausible involvement of  $\alpha$ -synuclein in a variety of neurodegenerative diseases stems from its presence as modified aggregates in affected neurons. Aggregation of this protein has been attributed to several potential factors including SNCA gene mutation, decreased rate of degradation or possible alteration of  $\alpha$ -synuclein, such as truncations, missense mutations or chemical modifications by oxidative reactions. Consistent evidence have shown that mutations in the SNCA gene cause problems with movement and balance similar to those associated with PD. Changes in the promoter and five-point mutations in the coding sequence for  $\alpha$ -synuclein have been found in SNCA gene. These mutations are clustered in the N terminus of the protein, indicating that they interfere with a role played by the N terminus of the protein in its normal cellular role. One type of mutation includes changes in a single amino acid where alanine could be replaced with threonine at protein position 53 or with proline at position 30. These amino acid changes cause the  $\alpha$ -synuclein protein to misfold and aggregate. Another type of mutation could be caused by duplication or even triplication of one of the two SNCA genes in each cell, leading to an excess formation of  $\alpha$ -synuclein. Consequently, the accumulation of the misfolded protein becomes a key player in the identified pathology of PD, capable of impairing the normal function of affected neurons and disrupting the regulation of DA. Many SNCA-based experimental models of PD have been developed in order to gain a better insight into the role of SNCA in this disorder pathogenesis. Recent *in vivo* findings [23] have indicated that SNCA downregulation might have a protective effect on dopaminergic neurons. It was found that silencing human (h)SNCA gene with mir30-hSNCA (microRNA30 transcript) in DA terminals in the striatum can reduce the motor deficit observed in hSNCA-expressing rats and protect against the loss of SN

dopaminergic neurons. However, attempts to silence hSNCA gene with a short hairpin (sh)RNA in rat SN have been shown to protect against a hSNCA-induced forelimb deficit, but not DA neuron loss [24]. In addition, high levels of shRNA-SNCA were found to be toxic to DA neurons, whereas adjacent neurons subject to lower levels were protected by hSNCA gene silencing. Collectively, these observations suggest that hSNCA gene silencing may be used as a new therapeutic tool to control behavioral deficits in PD.

### 4. $\alpha$ -Synuclein as a PD biomarker?

Identification of PD has been largely based on clinical diagnostic criteria, which in some cases presents some limitations since degeneration of dopaminergic neurons precedes the onset of symptoms. Considering the current attempts toward the development of new therapeutic strategies, early and accurate diagnosis of PD is consequential. Clinical biomarkers are significant predictive tools that can be used in the early stages of PD and even to differentiate between different PD subtypes [25,26]. One ideal biomarker that is closely associated with PD pathophysiology is  $\alpha$ -synuclein protein. Several clinical studies have explored the potential use of this protein as a recognized PD biomarker in cerebrospinal fluid (CSF) [27] and measured its levels using ELISA as well as immunoblotting and immunoprecipitation with specific antibodies [28]. The findings revealed that the level of oligomeric  $\alpha$ -synuclein increases significantly in PD patients, whereas the total  $\alpha$ -synuclein and phosphorylated  $\alpha$ -synuclein measured in the CSF of PD patients and controls show no significant changes due to the low sensitivity of immunoprecipitation studies. Considering the numerous advantages of using biomarkers, researchers have started measuring  $\alpha$ -synuclein deposition in peripheral organs of patients with PD. Wang *et al.* [29] have reported an increased level of  $\alpha$ -synuclein deposited in the cutaneous autonomic fibers of patients with PD without affecting sensory fibers. This explains the occurrence of autonomic dysfunction in patients with advanced PD [30]. Despite the practicality and advantages of biomarkers as valuable clinical prognostic tools, detecting them in other peripheral tissues could be challenging and remains to be determined [31].

### 5. Targeting $\alpha$ -synuclein in synucleinopathies

$\alpha$ -Synucleinopathies is a major class of neurodegenerative disease characterized by the aberrant expression of SNCA gene leading to the formation of abnormal fibrillar aggregates of  $\alpha$ -synuclein protein in the cytoplasm of selective populations of neurons and neuroglial cells. Patients with clinical manifestation of synucleinopathy show a chronic and progressive decline in motor and cognitive functions, depending on the severity of the disorder and the spread of the lesions. As  $\alpha$ -synuclein is closely linked to PD pathogenesis, researchers have focused their attention on targeting this protein for

therapeutic intervention. Recent studies have shown that targeting  $\alpha$ -synuclein protein, its signaling pathways or its gene expression is challenging but effective in modifying motor behavioral deficits and physiological dysfunction associated with synucleinopathies. Moreover, many novel compounds were identified as a viable therapeutic strategy to inhibit or reverse the aggregation process [32]. Using a combination of experimental and computational techniques, Tóth *et al.* [33] have identified a small-molecule drug-like phenyl-sulfonamide compound (ELN484228) that has the ability to bind monomeric  $\alpha$ -synuclein and consequently reduce its recruitment to the phagocytic cup and to mature synapses. As a result, this molecule has been shown to rescue the  $\alpha$ -synuclein-induced disruption of vesicle trafficking and loss of dopaminergic neurons. These findings suggest that altering the properties of  $\alpha$ -synuclein could have potential therapeutic benefits for combating PD and other neurodegenerative disorder. Another approach for targeting this protein is to reduce the Ser129 phosphorylation by inhibiting the relevant kinases. The phosphorylation appears to play a key role in the formation of fibrillar aggregates that underlie the development of PD and related synucleinopathies since it is evident that increased phosphorylation of  $\alpha$ -synuclein highly correlates with its aggregation and toxic buildup in neurons. Equally important is the implications of genetic missense mutations in the kinases, PTEN-induced putative kinase 1 and leucine-rich repeat kinase 2, in the development of PD [34,35]. A number of other kinases and phosphatases are still emerging as having potential roles in the pathogenesis of PD pathogenesis. Many kinase inhibitors could be used to reduce protein phosphorylation. However, this approach presents a potential limitation since several kinases are capable of phosphorylating  $\alpha$ -synuclein. For instance, Polo-like kinases and other G-protein-coupled receptor kinases convert  $\alpha$ -synuclein into phospho-Ser129  $\alpha$ -synuclein, the modified form of  $\alpha$ -synuclein that appears most frequently within PD pathological inclusions. This elevated level of phosphorylated  $\alpha$ -synuclein persists despite the pharmacologic inhibition of one of the other kinases.

An alternative strategy for decreasing  $\alpha$ -synuclein phosphorylation by enhancement of protein dephosphorylation has also been proposed by Braithwaite *et al.* [36]. The results of this study demonstrated that protein phosphatase 2A (PP2A) is the primary enzyme involved in phospho-Ser129  $\alpha$ -synuclein dephosphorylation. It has also been revealed that treatment with eicosanoyl-5-hydroxytryptamide, an enhancer of PP2A activity, was effective in decreasing the levels of phospho-Ser129  $\alpha$ -synuclein in the mouse brain [37].

Collectively, these studies suggest that modulation of  $\alpha$ -synuclein phosphorylation holds promise as a viable strategy for disease-modifying therapeutic interventions. Since phosphorylation of  $\alpha$ -synuclein might yield undesirable and complex cellular effects, it remains to be studied.

## 6. Immunotherapy as a new paradigm for PD treatment

It is a widely held view that many neurodegenerative diseases, termed synucleinopathies, can arise due to short oligomeric forms of  $\alpha$ -synuclein, which can be termed as neurotoxic. More recently, attention has been focused on the assimilation of  $\alpha$ -synuclein oligomers into neighboring cells [38] via propagation through a prion-like misfolding [39]. Therefore, the mechanisms of  $\alpha$ -synuclein-induced neurodegeneration can be related either to the oligomer toxicity or to the propagation and prion-like behavior of the small aggregates. Recently, there have been several reports strongly supporting the prion-like spreading of misfolded  $\alpha$ -synuclein [40-43]; some investigators however, have reported alternative explanations and interpretations for these findings. [44]. Therefore, an understanding of the mechanisms involved will have important consequences for immunotherapy. There has been increased interest in the development of immunotherapies, focused on the clearance of these aggregates and oligomers, as they are believed to be critical components in the neurodegeneration pathways [45,46]. For a while, Alzheimer's disease (AD) has been the focus of many immunotherapeutic studies, targeting extracellular protein aggregates, for example, A $\beta$ . Meanwhile, less attention has been paid to target proteins such as  $\alpha$ -synuclein and tau in PD and other neurodegenerative disorders. This has been mainly due to the fact that the A $\beta$  protein in AD is an extracellular molecule [47] that circulates in the blood and is easily recognized by antibodies. As indicated above, the fact that toxic forms of oligomeric versions of  $\alpha$ -synuclein can penetrate and accumulate in the plasma membrane and that they can be secreted and propagate extracellularly has provided a clear rationale for immunotherapy [45,48] and prompted the design of oligomer-specific antibodies [49].

Immunotherapeutic strategies, currently, are being considered as disease-modifying treatments for  $\alpha$ -synucleinopathies. Several early reports have shown that preclinical studies have been successful in clearing intraneuronal  $\alpha$ -synuclein aggregates using immunotherapy, also for stimulating or restoring the ability of the immune system to fight the disease [46,50-52].

Immunization against  $\alpha$ -synuclein can occur in one of two forms, active or passive immunity. In active immunity, the immune system is stimulated to produce antibodies against  $\alpha$ -synuclein aggregates and in passive immunization anti- $\alpha$ -synuclein antibodies are given to the patients. The rationale for immunization against  $\alpha$ -synuclein aggregates is to clear these neurotoxic substances and also inhibit neuron to neuron propagation [53]. Furthermore, in one study, it was reported that immunization resulted in the reduction of functional deficits and reversal of neurodegeneration, suggesting that the effect was promoted by microglial-mediated clearance of extracellular  $\alpha$ -synuclein [53]. A more recent study demonstrated that  $\alpha$ -synuclein mAbs blocked  $\alpha$ -synuclein entry

and cell-to-cell transfer of  $\alpha$ -synuclein pathology in primary neurons, thereby abolishing propagation and transmission of  $\alpha$ -synuclein pathology to other neurons [54]. An anti- $\alpha$ -synuclein protofibril-selective mAb (mAb47) was evaluated in another study, using  $\alpha$ -synuclein transgenic mice with motor dysfunction symptoms and increased display of  $\alpha$ -synuclein protofibrils in the CNS [55]. The reported results showed that injections of mAb47 resulted in significantly lower levels of both soluble and membrane-associated protofibrils of  $\alpha$ -synuclein. There was also a reduced motor dysfunction in these animals [55]. In a more recent study, passive immunization against the truncated C terminus of  $\alpha$ -synuclein was performed using the mThy1- $\alpha$ -synuclein transgenic mouse which resembles the motor deficits of PD; mice were immunized with the new mAbs 1H7, 5C1 or 5D12, all directed against the C terminus of  $\alpha$ -synuclein. Treatment with these antibodies attenuated synaptic and axonal pathology, rescued the loss of tyrosine hydroxylase fibers in striatum and improved motor and memory deficits [56].

A potential major stumbling block for the possible use of antibodies in targeting  $\alpha$ -synuclein is the presence of the blood-brain barrier, which might restrict the entry of antibodies for the purpose of clearing the toxic protein. However, novel approaches of delivery and antibody engineering can significantly increase the success of the treatment [57]. Recently, there have been reports about the use of intrabodies for targeting misfolded proteins in synucleinopathies and other disorders. Intrabodies are antibody fragments that have been engineered to be expressed intracellularly and they can be directed toward specific target antigens present in various subcellular locations [58-60]. There are also reports of the potential use of grafted amyloid-motif antibodies (gamma-bodies) that inhibit fibril assembly of peptides from A $\beta$  and  $\alpha$ -synuclein and other pathologic peptides [56,61].

Immunotherapy targeting  $\alpha$ -synuclein has evolved as a potential therapeutic strategy for neurodegenerative diseases, such as PD, and initial studies on cellular and animal models have shown promising results, but it still remains challenging as underlying mechanisms are not fully understood and further work is required for it to have important clinical use.

## 7. $\alpha$ -Synuclein-induced inflammation in neurodegenerative diseases

Immune response activation within the CNS is a prominent pathological feature of neurodegenerative diseases and an important contributor to neuronal damage. Inflammatory responses are typically manifested by increased expression of cytokines and other inflammatory mediators derived from reactive glial cells. Various animal models of PD have shown that neuroinflammation is not only a triggering event but also a process that accelerates the progressive loss of nigral dopaminergic neurons. Considerable research is currently directed on targeting proinflammatory mediators, such as cytokines and the transcription factors that regulate their expression, in an

effort to identify a possible novel therapeutic approaches for the treatment of inflammation-associated neurodegeneration [62]. According to recent work, in PD pathology, microglia-induced neurotoxicity and neuroinflammation have gained significant amount of attention [63], and it has been suggested that they play an active role in the symptoms and progression of the disease [64-66]. Interestingly, recent studies have demonstrated that dopaminergic neurodegeneration induced by misfolded  $\alpha$ -synuclein is aggravated by microglial activation through  $\alpha$ -synuclein phagocytosis and release of reactive oxygen radicals and cytokines [67]. It is suggested that  $\alpha$ -synuclein released from damaged dopaminergic neurons triggers the activation of microglia and promotes the release of proinflammatory mediators, thus leading to chronic and progressive dopaminergic neural degeneration associated with PD [68,69]. However, the relationship between microglial-induced neuroinflammation and PD-associated  $\alpha$ -synuclein aggregation remains elusive. Several recent studies have shed the light on the receptor mechanisms and signaling pathways involved in this process. A recent report has indicated that Toll-like receptor (TLR) 4 is required for  $\alpha$ -synuclein-dependent activation of microglia [63,70]. Similarly, when microglial cells were exposed to  $\alpha$ -synuclein that is released from neurons, TLR2 was found to be involved in  $\alpha$ -synuclein-induced microglia activation [67]. Release of IL-1 $\beta$  from monocytes by  $\alpha$ -synuclein fibrils has also been documented and shown to be mediated via TLR2 [71]. Additionally, the microglia exhibits an ability to detect misfolded  $\alpha$ -synuclein, resulting in an increased neurotoxicity through the production of reactive oxygen species and proinflammatory cytokines [72,73]. Furthermore,  $\alpha$ -synuclein can activate components of both the innate and adaptive immune systems in PD, and such interactions have been shown to modify the pathological processes in animal models of PD [74,75]. Even though the role of inflammation in the pathogenesis of PD remains unclear, overwhelming evidence suggests that dysregulated inflammation can be targeted for therapeutic intervention [76,77]. Based on the evidence that  $\alpha$ -synuclein fibrillar aggregates may be a key initiator of neuroinflammation, research studies are now directed toward understanding the mechanisms and pathways involved. New reports have indicated that many important brain functions depend on appropriate astrocyte-neuron interactions, and that a dysfunction or failure in this process might lead to pathological situations [78]. In PD, it has been shown that astrocytes play an important role in the initiation and progression of the disease [79,80]. For example, transfer of  $\alpha$ -synuclein to astrocytes causes inflammatory responses in synucleinopathies [79]. Furthermore, loss of DA neurons in the SN pars compacta is associated with an increased number of activated astrocytes [81] and moreover, the distribution of  $\alpha$ -synuclein-positive astrocytes parallels the distribution of LBs [82]. Therefore, understanding the role of astrocytes in neuroinflammation and pathogenesis of PD will be an important point in novel therapeutic approaches for the treatment of PD.

In a recently developed animal model of neuroinflammation [83], the nanopeptide PAT, a thymulin analog, was shown to target inflammation by downregulating the proinflammatory cytokines in the brain [84]. Based on these results, current work is being conducted on the efficacy of this peptide in animal models of PD and AD. Targeting neuroinflammation to reduce the pathological changes and nonmotor symptoms has been shown to have positive effects in different animal models of PD. It has been reported that soluble TNF, a proinflammatory cell-signaling protein, is required for robust endotoxin- or neurotoxin-induced nigral DA neuron degeneration [85]. Peripherally administered TNF inhibitor (XPro<sup>®</sup>1595) in an animal model of PD resulted in an attenuation of neuroinflammation and significant reduction in loss of nigral dopaminergic neurons [86]. Other studies using the same antagonist (FK506, also named tacrolimus) have demonstrated that the neuroprotective effects of this substance included reduction of  $\alpha$ -synuclein aggregation and associated neuronal cell death in cell cultures [87]. Furthermore, FK506 also reduced neuroinflammation and dopaminergic neurodegeneration in an  $\alpha$ -synuclein-based rat model of PD [88]. Glucagon-like peptide 1 (GLP-1) and the longer half-life GLP-1-like peptide exendin-4 (EX-4) have also been used. It is now being investigated for therapeutic use in PD [89,90]. Even though the precise mode of action of these peptides is not known, it is believed that they possess anti-inflammatory actions. Collectively, these findings suggest that the neuroinflammatory processes associated with PD not only involve the central immune system but also implicate the peripheral immune system.

In the cytokine network, IL-10 is considered to be a potent anti-inflammatory mediator, which is endogenously released by immune cells and glia as a negative feedback regulatory process [81,91]. IL-10-based therapeutic strategies for the treatment of neurodegenerative diseases are now being considered by several investigators [92]. In a recent study, the effect of a vector-containing cDNA for human IL-10 (AAV2-hIL-10) was investigated in a murine MPTP model of PD. Reported data suggested that the transfer of AAV2-hIL-10 into the striatum may play a neuroprotective role in the mouse MPTP model of PD and these effects are mediated by the anti-inflammatory action of IL-10 [93]. More recently, attention has been focused on the role of galectin-3 in microglial activation induced by exposure to  $\alpha$ -synuclein aggregates. Galectin-3 is a protein that specifically binds  $\beta$ -galactoside sugars, but most importantly, it participates in proinflammatory signaling pathways. It plays a dual role as a proinflammatory and anti-inflammatory substance depending on the cell type involved. *In vitro* studies using galectin-3 inhibitors and genetic ablation have confirmed the contribution of this protein to microglial activation induced by  $\alpha$ -synuclein and resulted in significant reduction of TNF- $\alpha$ , IL-12, inducible nitric oxide synthase and IL-1B levels [94]. Even though interest in anti-inflammatory approaches for the treatment of neurodegeneration has gathered a significant pace, additional research and clinical trials remain necessary to extend

this amassed knowledge and to develop better treatments for PD patients.

## 8. Expert opinion

PD is the second most common debilitating neurodegenerative disorder affecting ~ 2% of the population over the age of 60 and the chance of developing PD usually increases with age. Despite there being improvement in symptoms with current therapeutic strategies targeting DA receptors, sometimes the treatment is rendered ineffective in some patients at advanced stages. There is, therefore, an urgent need for a paradigm shift in therapeutic management and discoveries of novel and more effective treatments that prevent the initiation and stop the progression of the disease. Great attention has been given to the complex role of  $\alpha$ -synuclein protein in the pathophysiology of familial and sporadic cases of PD. The presence of  $\alpha$ -synuclein-containing insoluble cytoplasmic aggregates as a major protein constituent of LBs has been shown to play a crucial role not just in initiating but also in spreading the pathological process in PD. Mounting evidence suggests that  $\alpha$ -synuclein deposited in a hyperphosphorylated form with  $\beta$ -sheet-rich, fibrillar structure may self-propagate and spread gradually between interconnected brain regions by a cell-to-cell transmission mechanism causing accumulations of pathological  $\alpha$ -synuclein in different brain regions. This notion has been supported by a recent study [95] showing that inoculations of PD-derived LB extracts into the SN or striatum of both mice and monkeys result in gradual nigrostriatal neurodegeneration starting at striatal dopaminergic terminals. The injected exogenous human  $\alpha$ -synuclein was rapidly taken and deposited within the SN neurons and other anatomically connected neurons. Interestingly, for the LB to induce degeneration, the presence of human  $\alpha$ -synuclein as well as neuronal expression of this protein is required, indicating the pathogenicity of  $\alpha$ -synuclein contained in PD-derived LB. The mechanisms of  $\alpha$ -synuclein-induced neurodegeneration can be linked either to the oligomer toxicity or to the prion-like behavior of this misfolded protein. There is an emerging interest in the development of targeted immunotherapies, focused on removing the aggregated  $\alpha$ -synuclein oligomers, as they are believed to be key components in the neurodegeneration pathways [45,46].

However, one limitation of immunotherapies against misfolded proteins is the assumption that during the degenerative cellular states, the neuronal mechanisms that normally handle misfolded proteins will rapidly recover and respond adequately to further protein aggregation. This actually occurs *in vivo* over a period of hours [96]. McKinnon and Tabrizi [97] have observed prevalent changes in proteostasis in various models of protein aggregation, particularly in one of the most aggressive forms of neurodegeneration that is triggered by template-induced misfolding of prions. Effective animal models of prion propagation have demonstrated that the misfolded species can block the proteasome and downregulate protein translation.

Despite the limitations of immunotherapy targeting  $\alpha$ -synuclein, it is still considered a potential therapeutic strategy for neurodegenerative diseases, such as PD. Preliminary studies using cellular and animal models have shown promising results, but challenges remain to optimize the design of an effective treatment while awaiting further studies to reveal critical molecular pathways involved in the aggregation and folding of  $\alpha$ -synuclein.

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## Declaration of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents, received or pending, or royalties.

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