**Alpha-Synuclein Acts Like a Prion-Like Protein in Parkinson’s Disease**

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**Objectives:** The aim of this presentation is to critically examine whether alpha-synuclein behaves in a prion-like fashion in Parkinson’s disease (PD) and promotes the spread of neuropathology between different parts of the nervous system.

**Methods:** We used different Lewy body markers to stain post-mortem brains from several PD patients who had undergone intracerebral dopamine neuron transplantation more than one decade before death. Further, we have utilised cell cultures and animal models to examine whether alpha-synuclein can transfer from one cell to another and can seed protein aggregation in the recipient cell through a prion-like mechanism.

**Results:** Lewy pathology develops in dopamine neurons grafted to PD patients. The alpha-synuclein aggregates are ubiquitinated, thioflavin S-positive and stain for the P129S post-translational modification. These observations might explain why Lewy pathology seems to spread progressively following a stereotypical pattern (Braak stages 1-6) in the PD brain. Numerous subsequent studies in experimental models have shown that alpha-synuclein transfers between cells, and is taken up by neurons, oligodendrocytes, astrocytes and microglia. Some of the secreted alpha-synuclein is associated with exosomes. A significant portion of extracellular alpha-synuclein is taken up by neighbouring cells via endocytosis. Transferred alpha-synuclein can associate with endogenous alpha-synuclein. Recent animal experiments show that alpha-synuclein neuropathology propagates from one brain region to another.

**Conclusions:** Emerging evidence supports the idea that alpha-synuclein behaves in a prion-like fashion in PD. Deeper understanding of the prion-like behaviour of alpha-synuclein might lead to the identification of novel molecular targets for therapies that slow the progression of PD.
DISSECTING THE ROLE OF PATHOLOGICAL PROTEINS IN PARKINSON’S DISEASE PROGRESSION

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Objectives: Parkinson’s disease (PD) is characterized by the accumulation and spreading of misfolded alpha-synuclein in the brain. Other pathological proteins, including amyloid-beta, and microglia activation contribute to PD progression. Here, we aimed to dissect the role of pathological proteins and to study the sequence of cellular mechanisms involved in PD progression.

Methods: The load of pathological proteins, neurodegeneration and microglial activation was quantified in the brainstem, hippocampus and olfactory bulb of patients with incidental Lewy body disease (iLBD) and PD and age-matched controls. Oligomeric and protofibrillar alpha-synuclein were studied using novel conformation-specific antibodies. Multi-region transcriptome analysis was included to study the sequence of pathological and cellular events in donors with Braak PD stages 0 to 6.

Results: Oligomeric alpha-synuclein was most profound in synapses and small neurites, whereas protofibrillar alpha-synuclein was observed mainly in Lewy bodies. Alpha-synuclein pathology was associated with neuronal loss and microglia activation in iLBD and PD. Cortical amyloid-beta and tau pathology was limited in iLBD but contributed to the onset of dementia in PD. Functional pathway analysis revealed dysregulation of ceramide and sphingolipid metabolism, synaptic vesicle trafficking and lysosomal homeostasis in early-stage PD.

Conclusions: We showed that neurodegeneration and microglia activation are associated with alpha-synuclein pathology during PD progression. Sphingolipid, lysosomal and synaptic dysfunction may contribute to the proteinopathy in early-stage PD. Our combined histopathological and transcriptome multi-region analysis provides a model for the sequence of pathological events in PD and may be instrumental for defining diagnostic and prognostic biomarkers and drug targets for PD.
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**BIOMARKERS FOR ALPHA-SYNUCLEIN THERAPEUTICAL TRIALS**

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**Objectives:** Alpha-synuclein is an attractive target for putative disease modification strategies in Parkinson’s disease, and a number of new therapeutic agents modulating alpha-synuclein are in clinical development. In order to design informative clinical trials for alpha-synuclein therapeutics, it will be imperative to use biomarkers of target engagement and target modulation. The goal of this presentation is to review biomarkers that may be useful in clinical trials targeting alpha-synuclein. Data will be presented from a number of studies analysing various forms of alpha-synuclein in human biospecimens, including spinal fluid, blood and peripheral tissue biopsy samples. Additionally, an alpha-synuclein PET ligand will be described.

**Methods:** Data will be presented from several observational biomarker studies that have measured alpha-synuclein protein and other related biomarkers using standard laboratory immunoanalysis and proteomic methods. Motor and non-motor data were collected across multiple centres using standardised clinical assessments.

**Results:** Across multiple studies, alpha-synuclein protein and transcript levels appear altered in patients with Parkinson’s disease compared with controls. Pre-analytical variables, including sample processing, and analytical variables, such as antibody batch, significantly impact the biomarker results.

**Conclusions:** It is unclear at this time whether these markers change with disease progression but still may prove valuable to measure changes occurring with an intervention. The utility of these markers will be determined by their reliability and consistency across subjects, and it is critical to use accessible and standardised assays and sample collection protocols.
Developing PRX002, a Passive Immunotherapy Monoclonal Antibody Targeting Alpha-Synuclein for Parkinson’s Disease

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Objectives: Abnormal accumulation of alpha-synuclein is a neuropathological hallmark in Parkinson’s disease (PD) and other progressive degenerative alpha-synucleinopathies. Converging lines of evidence strongly suggest that alpha-synuclein may play a central role in the pathophysiology of PD. Moreover, toxic alpha-synuclein species have been proposed to propagate through interconnected neurons and to initiate pathology through processes that can be intercepted by anti-alpha-synuclein antibodies. An overview of the non-clinical findings that led to the development of PRX002 as a potential immunotherapeutic agent for PD will be presented.

Methods: The efficacy of anti-alpha-synuclein antibodies targeting various regions of the protein was assessed in a cell-to-cell transmission model in vitro and in chronic transgenic mouse models.

Results: In vivo studies demonstrated that antibodies targeting different epitopes of alpha-synuclein had varied responses in reducing the neuronal accumulation of aggregates, protecting against synaptic loss and gliosis and reducing motor and cognitive behaviour deficits in two mouse models of alpha-synucleinopathy. General concordance of these data was observed in vitro, specifically in cell-to-cell transmission of alpha-synuclein and the cleavage of alpha-synuclein by calpain.

Conclusions: Non-clinical studies support the concept that targeting specific epitopes of alpha-synuclein with antibodies is suitable to testing the alpha-synuclein hypothesis in PD. Accordingly, clinical trials with PRX002 have been initiated.
THERAPEUTICAL APPROACHES FOR SYNUCLEONOPATHIES

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Objectives: Parkinson’s disease (PD), dementia with Lewy bodies (DLB) and multiple system atrophy (MSA) are neurodegenerative disorders of aging, characterised by progressive accumulation of alpha-synuclein. Alterations in the balance among clearance, synthesis and extracellular release/uptake of alpha-synuclein might play a central role in the pathogenesis.

Methods: Strategies directed at targeting toxic alpha-synuclein species have been developed in recent years, and they may be of therapeutical value at managing synucleonopathies.

Results: Research in animal models of synucleinopathy have shown that active and passive immunisation against alpha-synuclein reduces the propagation of alpha-synuclein from neuron to neuron and diminishes the accumulation of intracellular alpha-synuclein aggregates, possibly via direct neutralisation and clearance through autophagy and microglia engagement. Moreover, antibodies targeting particular epitopes of alpha-synuclein appear to be more effective in cell-based and animal models, resulting in improved behavioural performance, reduced neurodegeneration and inflammation. Recent studies have also explored targeting various alpha-synuclein species using specific monoclonal antibodies, single-chain antibodies that direct aggregates to lysosomal degradation and small compounds that affect alpha-synuclein oligomerisation and membrane interactions.

Conclusions: Non-clinical studies support the notion that targeting alpha-synuclein might be a viable option for the treatment of synucleonopathies and clinical trials of both active and passive immunotherapy have been initiated in PD patients.