

Alpha-Synuclein: A THERAPEUTIC TARGET AND POTENTIAL BIOMARKER IN PARKINSON'S DISEASE

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The story of alpha-synuclein and Parkinson's disease (PD) began just two decades ago, when relatively newer genetic techniques at that time identified a single mutation in the gene encoding alpha-synuclein in a large Southern European family affected by Parkinson's.¹ This led to the widespread acceptance of genetics as a causative factor in PD and the observation of alpha-synuclein as a major player in the disease when it was noted to be a main component of Lewy bodies (the pathological hallmark of PD). Later, other alpha-synuclein mutations and extra copies of the alpha-synuclein gene were discovered in additional families with Parkinson's, suggesting that several different mutations as well as gene overexpression might be linked to Parkinson's. Although searches for alpha-synuclein gene abnormalities have been largely negative in the broad population of people with sporadic PD, research focusing on alpha-synuclein in PD remains attractive.

Our current understanding of alpha-synuclein biology is that either increased levels of wild-type alpha-synuclein protein or mutations in the alpha-synuclein gene result in abnormal folding of the protein, which in turn leads to aggregation. More recently, abnormal alpha-synuclein has been shown to move from affected to unaffected cells, suggesting an extracellular mechanism of pathological spread. These observations have led to three main avenues for alpha-synuclein-based research and development in Parkinson's:

- » Developing methods to measure and image alpha-synuclein that could lead to biomarkers, enabling earlier

and more accurate diagnoses and accelerating therapeutic development

- » Blocking the production of intracellular alpha-synuclein aggregates to potentially slow disease progression
- » Scavenging extracellular excess or abnormal alpha-synuclein to possibly alter disease course or even lead to improvement in existing cellular function

Potential Pathogenic Mechanisms of Alpha-Synuclein

Alpha-synuclein is found throughout the body and is ubiquitously expressed in neurons.² Its precise function is unknown, but it is thought to be involved in the regulation of neurotransmitter release at the synapse.^{2,3} Alpha-synuclein normally maintains a soluble monomeric (unfolded) configuration but, for any number of reasons — genetic alterations, environmental toxins, cellular stresses, etc. — can misfold, aggregate into different soluble oligomeric forms and then clump into insoluble fibrils. Abnormal alpha-synuclein has been hypothesized to induce cellular toxicity through one or more mechanisms:

- » Proteasome or lysosome impairment, leading to intracellular accumulation of proteins (including misfolded alpha-synuclein) that are normally degraded through this pathway,
- » Mitochondrial structure or activity dysfunction, resulting in failure of cellular energy production,

- » Cellular membrane pore formation, allowing calcium influx that disturbs cellular homeostasis,
- » Generation of chronic endoplasmic reticulum dysfunction, which induces cell death, or
- » Interference with neuronal signaling at the synapse, negatively impacting dopamine release.^{2,3,4,5}

A Theory of Alpha-Synuclein Propagation

In addition to the above intracellular effects, some evidence supports a cell-to-cell transmission of alpha-synuclein in Parkinson's. Braak and colleagues found pathological alpha-synuclein aggregates throughout the brain and in the periphery;⁶ pre-clinical PD models have demonstrated movement of alpha-synuclein between cells; and two groups independently visualized Lewy bodies in grafted fetal dopaminergic neurons of Parkinson's patients at autopsy.^{7,8} The "prion-like" hypothesis of alpha-synuclein holds that non-native forms of alpha-synuclein are released from cells into the extracellular space and taken up by neighboring cells, where they induce protein misfolding and the eventual development of Lewy bodies.^{4,5} Along the way, an inflammatory response, replete with microglial activation and pro-inflammatory cytokines, also may occur.^{2,3} Questions remain on how alpha-synuclein pathology progresses throughout the brain and why that progression is different from patient to patient. It is important to note that while it looks like alpha-synuclein pathology can spread within the brain of the patient, there is no definitive evidence to date that PD can, either directly or indirectly, be transmitted from one person to another.⁵

Alpha-Synuclein Therapies in Clinical Testing

Interruption of alpha-synuclein as it moves between adjacent cells is an appealing target for potential disease modification. Equally valid therapeutic pursuits include interventions at the point of post-translational modification, misfolding and aggregation. The development pipeline is rich with treatments targeting each of these missteps and several are in early stages of clinical trials:

» A drug to inhibit formation of alpha-synuclein oligomers.

Neuropore Therapies, Inc. and UCB are collaborating on an oral small molecule (NPT200-11), which aims to prevent the formation of alpha-synuclein oligomers. After favorable preclinical work, the compound advanced to a Phase I clinical trial in 55 healthy control volunteers. Results demonstrated safety and tolerability of several doses.⁹

» Immunotherapies to remove aggregated alpha-synuclein.

The objective of immunotherapy — either passive or active — is to clear out extracellular alpha-synuclein in order to decrease or prevent cell-to-cell spread of pathology. Passive immunotherapy is the infusion of manufactured anti-alpha-synuclein monoclonal antibodies. The antibodies can be given in precise amounts and the treatment can be reduced or discontinued if adverse effects occur. But, the antibodies must be regularly administered to maintain levels and efficacy, and tolerance cannot be predicted.¹⁰ Active immunotherapy is analogous to immunization. In Parkinson's, it's the injection of a small fraction of synthetic alpha-synuclein to induce a person's immune system to generate antibodies against it. In contrast to passive immunotherapy, it may last longer and require fewer injections over time. However, it could invoke neuroinflammation, polyclonal antibodies (which could bind off target and cause side effects), and significantly varied antibody response among subjects, namely the elderly who are less likely to generate high titers.¹⁰

Promising preclinical research with both types of immunotherapy supported the ongoing clinical trials.

Passive Immunotherapy

Prothena Biosciences Inc., in conjunction with Roche, is testing a humanized anti-alpha-synuclein antibody (PRX002). In a Phase I, randomized, double-blind, placebo-controlled, single ascending dose study of 40 healthy subjects it was shown to be safe and well tolerated and it reduced free serum total alpha-synuclein levels up to 96%.¹¹ A second Phase I, randomized, double-blind, placebo-controlled, multiple ascending dose trial of approximately 60 PD patients has recently been completed. It assessed safety, tolerability, pharmacokinetics, immunogenicity and exploratory



biomarkers. In addition to the Prothena/Roche program, Biogen is investigating another monoclonal antibody (BIIB054) against alpha-synuclein. The Phase I randomized, double-blind, placebo-controlled, single-ascending dose trial is evaluating safety, tolerability, pharmacokinetics and immunogenicity in 66 healthy subjects and early-stage (less than five years) Parkinson's patients.

Active Immunotherapy

AFFIRis AG, an Austrian biotech company, is testing an alpha-synuclein vaccine (AFFITOPE® PDO1A). A Phase I safety trial, supported by The Michael J. Fox Foundation (MJFF), assessed two different vaccine doses given once a month for four months in 24 early-stage Parkinson's patients (Hoehn & Yahr stage I/II; diagnosed within the last four years) and eight healthy controls. The vaccine was deemed safe and well tolerated, and approximately fifty percent of the patients developed alpha-synuclein antibodies in the serum and cerebrospinal fluid (CSF).¹² A follow-up "booster" study, also funded by MJFF, evaluated the safety, tolerability, and immunological and clinical activity of one administration of two different doses of a "booster" immunization. The booster was safe and well tolerated; only injection site reactions were more common in the treatment group. Eighty-six percent (19 of 22) vaccinated patients generated an immune response; of these responders, sixty-three percent (12 of 19) produced alpha-synuclein-specific antibodies. Preliminary observations revealed that eight of the 19 responders (42%) did not require an increase in dopaminergic PD medication during the observational study period (three years on average per participant), and five of these eight (63%) had stable UPDRS III scores at study conclusion.¹³ A "reboost" study, also funded by MJFF, in which volunteers will be given a second boost vaccination, has finished recruitment. This trial will evaluate long-term safety, immunologic and clinical response; results are expected in mid-2017.

Challenges in the Development of Alpha-Synuclein Therapies

With so many agents moving forward simultaneously, there is reason to be hopeful about a potential alpha-synuclein-based disease-modifying therapy. Any neurological drug has a

convoluted road to development, though, and alpha-synuclein-targeted therapies likely are no exception. In learning about and overcoming the hurdles related to alpha-synuclein, however, we're getting a better understanding of the disease, and moving closer to possible disease modifying therapies and a promising biomarker.

» Immunotherapy raises a unique set of challenges.

Antibodies are big molecules so whether they can cross the blood brain barrier in sufficient quantities to make an appreciable impact is one concern. Alzheimer's trials, however, have proven this feat is achievable and advances in drug delivery may be able to utilize blood brain barrier receptors to shuttle antibodies across, thereby improving antibody transport.² Another uncertainty relates to antibodies' activities once they gain access to the central nervous system. Do they bind to the toxic form(s) of alpha-synuclein? And, since immunotherapy doesn't get at intracellular alpha-synuclein, which is where the majority resides, does immunotherapy exert a sufficient effect? Future efforts may need to incorporate viral-vector-mediated and other delivery techniques to reach alpha-synuclein inside the cells.²

» The toxic species of alpha-synuclein is unclear.

Soluble oligomers are the most likely harmful conformation and, as such, are the most often targeted with current therapeutics. Still, any or all of the abnormal forms of alpha-synuclein could be the actual offender. Researchers are working to pinpoint the pathological species, and MJFF is funding a number of these endeavors.

» Normal role(s) and levels of alpha-synuclein are unknown.

As therapies affect alpha-synuclein, it's imperative they don't impede normal function or reduce levels below the lower limit of normal. Great strides in understanding the protein's physiological role have been made but its full function(s) and normal levels have not yet been elucidated. With continued trials and better quality assays, we will learn this critical information, which will help advance therapeutic development.

» Assays of alpha-synuclein are either not available or standardized/validated.

Creating consistent measurement processes and standard values for peripheral (e.g., serum and CSF) alpha-synuclein assays will make



reporting of trial results and comparisons across them easier. Studies have demonstrated that PD patients have lower total alpha-synuclein levels in the CSF but oligomeric or pathological levels cannot yet be reliably measured.² An early safety trial of passive immunotherapy measured total serum alpha-synuclein levels but the clinical significance of this is not yet known.¹¹ MJFF is funding research into the validation of alpha-synuclein biofluid and tissue assays. Increasing the specificities of these tests will allow for more informative, dependable measures and more refined therapeutic approaches.

» **Alpha-synuclein cannot be imaged *in vivo*.** Rather than using surrogate peripheral measures, it would be preferable to directly visualize alpha-synuclein load in the brain. An alpha-synuclein imaging ligand could confirm the diagnosis of Parkinson's by assessing the presence and distribution of alpha-synuclein in the brain, ensure the right patients are enrolling in clinical trials and objectively measure therapeutic impact. An imaging agent would markedly accelerate ongoing therapeutic endeavors. To

fuel these efforts, MJFF leads a collaboration of investigators called the Alpha-Synuclein Imaging Consortium and recently announced an Alpha-synuclein Imaging Prize, a \$2 million award for the first team to create a selective alpha-synuclein PET tracer.

In lieu of or in addition to imaging alpha-synuclein in the brain, peripheral measurements of the protein may serve as a biomarker. Efforts are ongoing to characterize alpha-synuclein levels in various body regions (CSF, serum, colon, skin and submandibular gland) in people at all disease stages.

These hurdles are not insurmountable. They should not be viewed as deterrents to alpha-synuclein-based therapies but rather as steps that, as they are addressed, move therapeutic development and biomarker validation forward in parallel. With so many potential disease-modifying therapies in clinical testing, we are closer than ever to realizing a treatment that could alter the course of disease. For many people with Parkinson's and their providers, this can be a source of great hope and optimism.

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Article reviewed by Kuldip Dave, PhD, Director of Research Programs at the Michael J. Fox Foundation for Parkinson's Research.

The Michael J. Fox Foundation is the largest nonprofit funder of Parkinson's disease research worldwide. The Foundation is dedicated to finding a cure for Parkinson's disease through an aggressively funded research agenda and to ensuring the development of improved therapies for those living with Parkinson's today. Because patients are vital partners in this process, the Foundation works to mobilize volunteer engagement in research by providing education and direct research-related services to Parkinson's clinicians, researchers, patients and families.



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*For a review of DLB, see the article entitled "Dementia with Lewy Bodies: In the News and In the Clinic" in the January/February 2015 issue of Practical Neurology®, available at practicalneurology.com.

