UNTANGLING α-SYNUCLEIN

By Mark Zipkin, Staff Writer

The Michael J. Fox Foundation for Parkinson’s Research (MJFF) wants to settle the question of what form of α-synuclein drives the pathology of Parkinson’s disease. To make sure the data hold up in different labs using different methods, the Foundation is funding parallel projects with multiple teams. Its goal is to get answers within two years, but if it only comes away with better assays, the organization will still consider the money well spent.

Part of the problem in creating therapies against α-synuclein is that several different forms exist — including monomeric, oligomeric, glycosylated and phosphorylated species — but little is known about which ones contribute to the disease, according to Kuldip Dave, director of research programs at MJFF.

Aggregates of the protein, which constitute about 90% of the Lewy bodies that are hallmarks of PD, have been associated with the disease since the late 1990s. Despite that, the most advanced α-synuclein-based therapies to date are only in Phase I.

Affiris AG’s PD01A and PD03A vaccines, Roche’s RG7935 and the PRX002 immunotherapy from Roche and Prothena Corp. plc — all in Phase I — aim to disaggregate α-synuclein clumps. In addition, several preclinical compounds — including Prana Biotechnology Ltd’s PBT434, NeuroPhage Pharmaceuticals Inc’s NPT001 and ProteoTech Inc’s synuclere — are designed to prevent α-synuclein clumping.

Moreover, the α-synuclein aggregates found in PD are known to include nitrated forms of the protein. But dissecting how nitration drives aggregation has been challenging, because the protein can be nitrated at multiple sites, and methods for producing each nitrated form in a quantity sufficient for study have been lacking. Last week, a study in the Journal of the American Chemical Society reported a method for synthesizing several forms of nitrated α-synuclein on the milligram scale and examined their pro-aggregation properties in vitro — but the findings are still a long way from elucidating the role of α-synuclein nitration in PD.

“It’s clear that an increase in α-synuclein is involved with pathophysiology of Parkinson’s disease,” said Dave. “So our goal is to lower α-synuclein and target α-synuclein with very different strategies to lower overall levels, or lower the toxicity or pathology that’s associated with α-synuclein.”

He added, “Mouse knockout models where α-synuclein is completely absent do not show any negative effects. From the animal model standpoint, there isn’t anything obvious to worry about.”

But the bottom line, he said, is that “we don’t know α-synuclein’s normal function” and there are different species of the protein that are found in disease onset and progression.

Last month, the organization announced it is committing $2 million to the Species LEAPS (Linked Efforts to Accelerate Parkinson’s Solutions) program to address the question of which α-synuclein species drives disease pathology. In addition, MJFF launched the $2.2 million Assay LEAPS initiative last year to develop and validate assays for α-synuclein.

Members of the Species and Assay LEAPS consortia will also join other MJFF-funded researchers working on α-synuclein to form the Investigating Synuclein Consortium, in which the participants will share findings and tools to advance projects across the board.

Dave told BioCentury that to avoid problems of “lab A finds this and lab B finds that,” the Species LEAPS program will fund three projects in cross-validating teams that aim to characterize different species of α-synuclein and determine their effect on PD development. Ultimately, MJFF hopes to parlay the findings into new therapeutics or biomarkers for PD.

“If we know what that pathological species is, we can follow it to see whether that’s changing as the disease progresses to see if, not only are you having a beneficial effect on symptoms, but you’re actually changing the disease,” said Dave.
TEAM PLAYERS
The LEAPS approach is to establish parallel teams with some overlap in their projects. The teams will identify species with a pathological bearing on PD and then cross-validate results wherever possible. “We’re asking teams to come together and validate and cross-validate in the same tissue using the same or different techniques,” said Dave.

Other attempts have been made to identify or quantify the most important α-synuclein species in PD, but the data from individual labs has been difficult to replicate. Dave said technical issues were most likely the reason for the variation. For example, different groups use different tissues or tissues taken at different times post-mortem, employ different antibodies or analyze material with different mass spectrometric techniques.

Dave hopes the standardization and cross-validation can ensure the results are unambiguous at the end of the program. “We don’t want to be two or three years from now where each of the teams or sub-teams have come up with their own species, and none of them really match up to each other. For pharma and others, it would be tough to understand which species to go after therapeutically or as a biomarker,” he told BioCentury.

The teams will be in regular contact, which includes quarterly presentations to each other and MJFF.

The first team, led by Brit Mollenhauer, professor of neurology at the University of Göttingen, includes researchers from Bristol-Myers Squibb Co., Biogen Idec Inc., BioLegend Inc. and Quanterix Corp.

Dave said the Mollenhauer team “will do mass spec on over 200 CSF samples to identify the most prevalent species and ask, ‘Are they different between healthy and Parkinson’s disease patients?’ And then if they’re different, can we quantify them?”

He added that validation within the group is key. “One lab will run mass spec, and then send the same samples to another lab, and then they will run mass spec and see if they find exactly the same data. To us, that’s very important.”

If Mollenhauer’s team identifies a species that is more prevalent, her group will develop an assay at BioLegend Inc. “That assay would then be available through MJFF to the wider research community,” said Dave.

BioLegend acquired Covance Antibody Services Inc. in August 2014. Covance previously developed an α-synuclein ELISA assay with MJFF.

The second team is led by Henrik Zetterberg, professor of neurochemistry at the University of Gothenburg. Zetterberg’s group will also identify different species of α-synuclein but will focus on brain tissue instead of CSF. “They have a bank there that has brains from PD, Multiple System Atrophy (MSA) and controls, and will be comparing within their team and cross-validating,” said Dave.

The third group is also working with brain samples and is led by Dennis Selkoe, co-director of the Center for Neurologic Diseases at Brigham and Women’s Hospital. Dave said the group will “look at healthy controls, PD and some other synucleinopathies like dementia with Lewy bodies and MSA, to see if they can differentiate some of the species between synucleinopathies.”

After 18 months, the brain tissue teams will cross-validate their findings with blinded samples from the Arizona Parkinson’s Disease Consortium (APDC) that include healthy controls and brains from various stages of disease.

The LEAPS model came together in 2014 with the Assay LEAPS initiative that was designed to develop an assay for total α-synuclein, which would recognize all the different species of the protein. According to Dave, there are only about four assays
available that can measure total α-synuclein, but they are not standardized.

“There are other assays that were developed,” he said. “But they have different signal-to-noise ratios, you have to run tissues or CSF samples in different ways in these assays, and there’s really no standardization and cross-validation that has been done with a single assay.”

Dave sees the Assay LEAPS initiative as a good outcome for MJFF even if characterizing the specific species involved in PD turns out to be harder than anticipated.

“In the event we don’t identify specific species — maybe there isn’t a species that falls out of the Species LEAPS — we would have at least standardized total α-synuclein,” he told BioCentury.

COMPANIES AND INSTITUTIONS MENTIONED

Affiris AG, Vienna, Austria

Biogen Idec Inc. (NASDAQ:BIIB), Cambridge, Mass.

Bristol-Myers Squibb Co. (NYSE:BMY), New York, N.Y.

BioLegend Inc., San Diego, Calif.

The Michael J. Fox Foundation for Parkinson’s Research (MJFF), New York, N.Y.


Prana Biotechnology Ltd. (ASX:PBT; NASDAQ:PRAN), Parkville, Australia

Prothena Corp. plc (NASDAQ:PRTA), Dublin, Ireland

ProtoTech Inc. Kirkland, Wash.

Quanterix Corp., Cambridge, Mass.

Roche (SIX:ROG; OTCQX:RHHBY), Basel, Switzerland

University of Gothenburg, Gothenburg, Sweden

University of Göttingen, Göttingen, Germany

TARGETS AND COMPOUNDS

α-synuclein (SNCA)

REFERENCES