PARKINSON’S DISEASE BIOMARKER DEVELOPMENT: IMMUNOPRECIPITATION LC-MS/MS TO QUANTITATIVELY DETECT PHOSPHORYLATED ALPHA-SYNUCLEIN IN HUMAN CSF

M. Copeland¹, A. Xu¹, T. Turi¹, M.M. Mouradian², S.P. Braithwaite³, R.L. Martone¹

¹Biomarker Center of Excellence, Covance, Inc., Greenfield, IN, ²Center for Neurodegenerative and Neuroimmunologic Diseases, University of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School, Piscataway, ³Signum Biosciences, Monmouth Junction, NJ, USA

Introduction: Parkinson's disease (PD) is a global neurodegenerative disease characterized by progressive deterioration of motor function and cognition. Histopathologically, PD is characterized by the intracellular accumulation of alpha-synuclein (a-syn) in the form of Lewy Bodies and Lewy Neurites. Mutations in a-syn, as well as duplication and triplication of the wild-type locus cause autosomal dominant familial PD. a-Syn is subject to post translational modifications, including phosphorylation and nitration, which results in neurotoxic species. Assessment of phosphorylation status may provide a closer connection to disease progression than total a-syn levels alone. Although primarily an intracellular protein, a-syn is actively excreted by neurons, therefore providing an accessible pool in CSF for biomarker evaluation. Here, we report the development of immunoprecipitation (IP) LC-MS/MS methods to measure low-abundance phosphorylated forms of a-syn in human CSF compared to plasma for biomarker applications.

Aims: To assess feasibility of using CSF levels of phosphorylated a-syn as a biomarker for PD.

Methods: Phosphorylated species of a-syn (at S87, Y125, S129 and Y133) were immunoprecipitated from non-PD human CSF and plasma with phospho-specific antibodies. The immunoprecipitated protein was subjected to trypsin digestion and a unique signature peptide was analyzed by LC-MS/MS.

Results: All phospho-epitopes were readily detected in both CSF and plasma. Overall, CSF levels of phosphorylation were ~14% of plasma levels.

Conclusions: IP LC-MS/MS methodology of specific a-syn species may provide a novel biomarker approach for PD. This technique is readily applicable to other low abundance CSF analytes and provides sufficiently increased sensitivity for their detection.