1. PROJECT GOALS / FEASABILITY

A. BACKGROUND

Parkinson's Disease (PD) is a neurodegenerative disorder that primarily affects motor abilities of individuals over the age of 60.[1] PD presents with tremors, muscle rigidness, bradykinesia (slow body movements), poor balance and posture and loss of coordination. There can also be non-motor symptoms such as impairments in an individual's cognitive ability, sleep disorders, swallowing problems, emotional changes and/or depression, bladder and bowel.[1] Patients with PD are found to have decreased levels of the neurotransmitter called dopamine. In addition, they also have aggregation of a protein called alpha-synuclein (α-syn) in their brain cells. Although the cause of PD is still unknown, over-expression or high level of α-syn protein has been found to decrease the function of dopaminergic neurons, suggesting impairment of α-syn protein may lead to PD.[2]

MicroRNAs (miRNAs) are small RNA molecules that regulate many important biological processes, including turn-on or turn-off of specific gene expression in the cells. In the oncology field, abnormal expression of miRNAs has been identified to reflect the pathological status of patients and serve as potential biomarkers. In PD, it has been found that the expression of miRNA-34b and 34c is lower in brain tissues of PD patients and cell lines, compared with healthy controls.[3,4] Also, down-regulation of miRNA-34b/c has been shown to increase the expression of α-Syn.[4] Since there is no cure for PD, accurate monitoring of disease progression is needed to gauge old or new drug efficacy: PD may continue to progress with drug A but not with drug B; drug C slows or stops PD progression in patient X, but not in patient Y. However, to date, there is no proven laboratory test to monitor PD. Thus, there is important to develop PD progression biomarkers that are clinically relevant to enable more effective treatment.

B. GOALS

i) Specific Aim: Previously, [redacted] led a biomarker study to evaluate 4 PD-related miRNA biomarkers she identified[5] as potential PD progression biomarker (manuscript in preparation). Her recent preliminary study also showed that miRNA-34b/c can differentiate healthy controls from newly-diagnosed and advanced PD. Here, our specific aim is to evaluate the expression of miRNA-34b/c in sera of same PD patients at baseline (0 month; first diagnostic of PD) and endpoint (6-24 months of disease progression) as potential progression biomarkers for PD. Since reports have shown that patients with PD have lower expression levels of miRNA-34b/c compared with healthy controls, we hypothesize that miRNA-34b/c expression will be lower at the endpoint than baseline to reflect disease progression. This proof-of-concept study will demonstrate the feasibility of developing blood-based progression biomarkers to benefit PD patients.

ii) Research Strategies: We will analyze a total of 30 patient samples (15 baseline and 15 endpoint serum samples from same PD patients) from our on-going PD biomarker study (see attachment in Supplemental Section for an institutional review board approval). The sample came from the DATATOP (deprenyl and tocopherol antioxidative therapy of parkinsonism) cohort where patients underwent a clinical trial to test if deprenyl and/or tocopherol increased the amount of time before levodopa was necessary to manage symptoms of PD.[6] Total RNA that contains miRNAs will be extracted and quantified before performing quantitative real-time polymerase chain reaction (qRT-PCR). qRT-PCR allows measuring of miRNA expression using specific miRNA fluorescent probe, in this case, miRNA-34b/c probes. Since the scholar has not been working in [redacted] lab, initially, she will be taught basic molecular lab techniques (pipetting, dilution calculations, RNA quantification) and gradually trained on qRT-PCR

techniques and analysis. [redacted] will personally train the scholar to maximize the scholar's research experience.

C. PROJECTED TIMELINE

Date	Task (Scholar will be mentored by [redacted] for each task)
May 7 – May 27	Basic molecular lab. techniques training and, sample selection
May 28 – June 3	RNA (including miRNAs) extraction and quantification
June 4 – June 10	Pre-amplification of RNA containing miRNAs
June 11 – June 17	Reverse transcription of miRNA-34b/c
June 18 – July 14	Perform qRT-PCR for miRNA-34b/c expression
July 16 – August 4	qRT-PCR data analysis and interpretation, progress report

D. AREA OF EXPERTISE

[redacted] has been researching on miRNA-related biomarkers for PD using various molecular techniques since 2009 which led to 5 published manuscripts. Thus, we do not anticipate any technical or scientific difficulties performing and completing this MS3 project.

E. REFRENCES

[1]Dubois, B. & Pillon, B. (1996). Cognitive deficits in Parkinson's disease. *Journal of Neurology, 244*(1), 2-8.

[2]Venda, L. L., Cragg, S. J., Buchman, V. L., Wade-Martins, R. (2010). α-Synuclein and dopamine at the crossroads of Parkinson's disease. *Trends in Neuroscience, 33*(12), 559-568.

[3]Miñones-Moyano E., Porta S., Escaramís G., Rabionet R., Iraola S., Kagerbauer B., Espinosa-Parrilla Y., Ferrer I, Estivill X., Martí E. (2011). MicroRNA profiling of

Parkinson's disease brains identifies early downregulation of miR-34b/c which modulate mitochondrial function. *Hum Mol Genet. 20*(15), 3067-3078.

[4]Kabaria, S., Choi, D. C., Chaudhuri, A, D., Mouradian, M. M., Junn, E. (2015). Inhibition of miR-34b and miR-34c enhances α-synuclein expression in Parkinson's disease. *FEBS Letters, 589*(3), 391-325.

[5]Khoo S. K., Petillo D., Kang U. J., Resau J. H., Berryhill B., Linder J., Forsgren L., Neuman L. A., Tan A. C. (2012). Plasma-based circulating MicroRNA biomarkers for Parkinson's disease. *J Parkinsons Dis.* 2(4), 321-331.

[6]DATATOP: A Multicenter Controlled Clinical Trial in Early Parkinson's Disease Parkinson Study Group. *Arch Neurol.* 1989;46(10):1052-1060.

F. SUPPLEMENTAL SECTION

1) Itemized Budget

Title of Project:	miRNA-34b/c as disease progression biomarkers for Parkinson's disease
Student name:	[redacted]
Faculty mentor(s) name:	[redacted]

STIPENDS	
Student stipend[1]	[redacted]
Faculty stipend	[redacted]

PROJECT COSTS (please list items/services and estimated costs)[2]	
miRNA-34b/c qRT-PCR probes	[redacted]
TOTAL	[redacted]

FUNDING FROM OTHER SOURCES (list amount and source)[3]	