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TITLE: Circulating CNS Cell-Derived Extracellular Vesicle-Based Biomarkers to Identify Neurodegeneration and Glial Activation in Multiple Sclerosis

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Extensive synaptic loss is noted in MS brain and spinal cord samples and may independently drive disability progression. The mechanism of synaptic loss may be related to complement deposition and subsequent synaptic engulfment by phagocytic cells. Extracellular vesicles (EVs) are produced by all cells in the body and have both physiological and pathological roles. Neuronally-enriched (NEVs) and astrocyte-enriched (AEVs) EVs can be isolated from peripheral blood using immunoprecipitation for cell-specific markers. SPRINT-MS was a phase 2, randomized, placebo-controlled trial assessing the effect of ibudilast on several measures of tissue destruction in 255 participants with progressive MS. This trial included deep clinical and imaging phenotyping in addition to collection of biospecimens, making it an ideal cohort to evaluate the utility of these biomarkers. We will utilize plasma from the SPRINT-MS cohort to isolate NEVs and AEVs using an extensively validated method. We will then measure synaptic protein levels (synaptophysin and synaptopodin) in NEVs and complement component levels in AEVs. We will utilize mixed-effects models to examine both cross-sectional and longitudinal relationships between EV-based measures and clinical (EDSS, MSFC) and imaging (MRI and OCT) based measures and examine trajectories in the two treatment groups to determine whether these novel measures are good correlates for disease progression and monitoring of the effects of putative neuroprotective agents.

15. SUBJECT TERMS

Multiple Sclerosis, extracellular vesicles, synapses, astrocytes, complement

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1. Introduction:

MS is an autoimmune disease with inflammatory and neurodegenerative components. Besides axonal damage, extensive synaptic loss is noted in MS brain and spinal cord samples, and is even seen in grey matter areas without demyelination. Synaptic loss may independently drive disability progression, however there are currently no validated biomarkers to track this process. The mechanism of synaptic loss may be related to complement deposition and subsequent synaptic engulfment by phagocytic cells. Reactive neurotoxic astrocytes upregulate production of complement component C3 in several neurodegenerative disorders, including MS.

Extracellular vesicles (EVs) are produced by all cells in the body and have both physiological and pathological roles. Neuronally-enriched (NEVs) and astrocyte-enriched (AEVs) EVs can be isolated from peripheral blood (plasma or serum) using immunoprecipitation for cell-specific markers. Several neurological disease states show alterations in a range of biomarkers measured in NEVs and AEVs. We recently demonstrated that in MS patients, NEV synaptic protein levels were markedly reduced compared to controls. We also noted that AEVs in MS patients had elevated levels of multiple complement components compared to controls and AEV C1q levels were associated with greater disease severity and the presence of chronic active/ paramagnetic rim lesions on imaging. These results parallel findings of synaptic loss and glial activation noted in MS autopsy tissue.

SPRINT-MS was a phase 2, randomized, placebo-controlled trial assessing the effect of ibudilast on several measures of tissue destruction in 255 participants with progressive MS. This trial demonstrated a 48% reduction of brain atrophy in ibudilast-treated participants compared to placebo during the 96-week study period. Ibudilast is a phosphodiesterase-4 inhibitor that is thought to have neuroprotective properties related to direct neuronal effects and alterations in inflammatory glial cell activation. The SPRINT-MS trial included deep clinical and imaging phenotyping in addition to collection of biospecimens, making it an ideal cohort to evaluate the utility of these novel EV-based biomarkers.

We hypothesized that NEV synaptic protein levels provide a biomarker for neurodegeneration (specifically synaptic loss) in MS and mirror clinical and imaging metrics of disease progression, while AEV complement levels identify glial activation and predict subsequent neurodegeneration.

We will utilize plasma collected at baseline, 48, and 96 weeks from the SPRINT-MS cohort to isolate NEVs and AEVs using an extensively validated method. We will then measure synaptic protein levels (synaptophysin and synaptopodin) in NEVs and complement component levels in AEVs. We will utilize linear regression and mixed-effects models to examine both cross-sectional and longitudinal relationships between EV-based measures and clinical (EDSS, MSFC) and imaging (MRI and OCT) based measures and examine trajectories in the two treatment groups to determine whether these novel measures are good correlates for disease progression and monitoring of the effects of putative neuroprotective agents.

This research may identify novel biomarkers of neurodegeneration (NEV synaptic protein levels) and glial activation (AEV complement levels) that can be used to track disease progression. In the short-term, these biomarkers could identify MS patients at greater risk for disease progression and serve as outcomes for proof-of-concept trials of neuroprotective and reparative therapies. In the long-term, these biomarkers may have clinical applications including monitoring disease progression and treatment response in individuals with MS.

2. Key Words:

Multiple Sclerosis, extracellular vesicles, synapses, astrocytes, complement

3. Accomplishments:

What were the major goals of the project?

The major goals of the study are –

1. Determine whether NEV synaptic protein levels reflect disease status as measured by clinical and imaging metrics in SPRINT-MS trial participants.
2. Determine whether AEV complement levels predict neurodegeneration in progressive MS

Below is a list of the major tasks as listed in the SOW -

Aim 1
a. Major Task 1 – Obtain Regulatory Approvals
b. Major Task 2 – Coordinate Study Staff
c. Major Task 3 – Determine logistics for study
d. Major Task 4 – Measurement of biomarkers in NEVs
e. Major Task 5 – Data Analysis

Aim 2
a. Major Task 1 – Obtain supplies for AEV isolation and complement measurement
b. Major Task 2 – Measurement of biomarkers in AEVs
c. Major Task 3 – Data Analysis

What was accomplished under these goals?
The accomplishments of each stated tasks correspond with each bullet point above.

Under Aim 1.

A) **Obtain Regulatory Approvals** – We obtained approval from local IRB (Milestone Achieved) from the study and then from HRPO (Milestone Achieved). We also had to get approval from the Cleveland Clinic Foundation IRB which was received and then set up the MTA between CCF and JHU to allow for transfer of samples (Milestone achieved).

B) **Coordinate study staff** - We advertised for and hired a laboratory technician – Deepika Joshi, BS who then underwent extensive training in the methodology for isolation and characterization of various EV sub-populations from serum samples (Milestone Achieved). We also received assistance from the lab manager Yasmin Resto and recently have hired a new postdoctoral fellow Satheesh Kumar PhD who will help supervise and perform several aspects of EV subpopulation isolation and downstream assays.

C) **Determine logistics for study -** We identified freezer space for storage of samples at JHU and purchased some supplies for NEV and AEV isolation (Milestone Achieved). We also successfully transferred plasma samples from SPRINT MS trail from CCF to JHU and stored them in the space identified (Milestone Achieved).

D) **Measurement of biomarkers in NEVs** – We have begun isolation of NEVs from the SPRINT MS samples but have not completed this task at this time as scheduled (scheduled for month 12) and expect to complete this by the end of the year or early next year (Month 16-18). Following this we will begin the measurement of CD81 and synaptic markers in the NEVs using ELISAs.

E) **Data Analysis** – This will begin following the completion of biomarker measurement in NEVs as noted above.

Under Aim 2.

A) Obtain supplies for AEV isolation and complement measurement - We have purchased some supplies for AEV isolation (Milestone Achieved). We will also purchase CD81 ELISAs and complement component Luminex assays following completion of AEV isolation from all SPRINT MS samples.

B) Measurement of biomarkers in AEVs – We have begun isolation of AEVs from the SPRINT MS samples ahead of schedule (scheduled for month 18-23) and expect to complete this by the end of the year or early next year (Month 16-18). Following this we will begin the measurement of CD81 and complement components in AEVs.

C) Data Analysis – This will begin following the completion of biomarker measurement in AEVs as noted above.

What opportunities for training and professional development has the project provided?
The project has allowed for training of the new laboratory technician in isolation of EV subpopulations from plasma samples.

How were the results disseminated to communities of interest?
Nothing to report.

What do you plan to do during the next reporting period to accomplish the goals?
During the next reporting period, we plan to complete enrollment of the eligible participants and collect various study outcome measures as planned. We will plan to perform metabolomics and microbiome analyses – once all participants have completed the study intervention and will then proceed with data analysis and manuscript preparation.

4. Impact:

What was the impact on the development of the principal discipline(s) of the project?
Nothing to Report

What was the impact on other disciplines?
Nothing to Report

What was the impact on technology transfer?
Nothing to Report

What was the impact on society beyond science and technology?
Nothing to Report

5. Changes/Problems:

Changes in approach and reasons for change?
Since we have to thaw the plasma samples from the SPRINT MS samples to isolate NEVs, we reasoned that during this initial freeze-thaw we could also isolate AEVs at the same time preventing an extra round of freeze-thaw for the samples.

Actual or anticipated problems or delays and actions or plans to resolve them
Due to combining the isolation of NEV and AEV in

Changes that had a significant impact on expenditures
Nothing to Report

Significant changes in use or care of human subjects
Nothing to Report

6. Products:

Nothing to Report

7. Participants & Other Collaborating Organizations

What individuals worked on this project?

Name:	Pavan Bhargava
Project Role:	Principal Investigator
Nearest person month worked:	1.8
Contribution to Project:	Dr. Bhargava has performed work in the area of study management and oversight (including drafting/revising protocol and IRB documents, submission of documents to HRPO and training the research coordinator), obtaining samples and supplies for NEV and AEV isolation followed by supervision of technician to isolate EVs from the SPRINT MS samples.

Name:	Deepika Joshi
Project Role:	Laboratory Technician
Nearest person month worked:	12.0
Contribution to Project:	Ms. Joshi has performed work in the area of sample storage and NEV and AEV isolation.

Name:	Yasmin Resto
Project Role:	Laboratory Manager

Nearest person month worked: 3.0
Contribution to Project: Ms. Resto has performed work in the area of sample transfer, storage, inventory and ordering of supplies for NEV and AEV isolation.

Name: Dr. Satheesh Kumar
Project Role: Postdoctoral fellow
Nearest person month worked: 2.2
Contribution to Project: Ms. Joshi has performed work in the area of sample storage and NEV and AEV isolation.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
Nothing to report

What other organizations were involved as partners?
Cleveland Clinic Foundation

Name: Sarah Planchon-Pope
Project Role: Site PI
Nearest person month worked: 0.24
Contribution to Project: Ms. Planchon-Pope helped to obtain regulatory approval at CCF and in the transfer of samples from CCF to JHU.

Name: Devon Conway
Project Role: Site Co-Investigator
Nearest person month worked: 0.24
Contribution to Project: Dr. Conway helped to obtain regulatory approval at CCF and in the transfer of samples from CCF to JHU.

8. Special Reporting Requirements

Nothing to Report

9. Appendices

Nothing to Report