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TITLE: Prediction of Future Disability in MS Using Combined Novel MRI and Serological Markers

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

In this annual report, we detailed our activities in the dates since initial IRB approval (10/26/2019) with emphasis on activities since our last annual report. We have continued to contact and schedule patients for study visits, beginning chart review for patients who prefer to come later for their study visit. 60 patients have completed in-person study visits, 10 have completed phone visits, 4 are scheduled for upcoming visits, and 120 patients have been contacted overall. MRI data reprocessing and analysis has been completed for all subjects. Study visits are ongoing and chart reviews have been started. Given the delays in recruitment, we have requested and been approved for a one year no-cost extension which will allow us to complete the remaining study tasks.

15. SUBJECT TERMS

NONE LISTED

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1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

We will study novel MRI and serological markers (analyzed on previously collected imaging and serum samples) as predictors of future disability in Multiple Sclerosis (MS). MS is heterogenous, patients display a wide spectrum of long-term disability levels that are not completely foreseen by early disease activity but may be explained, in part, by intrinsic patient-specific differences in central nervous system tissue susceptibility to damage and intrinsic ability to repair. Novel MRI and serological biomarkers, such as Gradient Echo Plural Contrast Imaging (GEPCI) and serum neurofilament light chains (NfL), may better reflect the early neurodegeneration in MS. We wish to determine if these novel biomarkers, separately and in combination, can be used as better predictors of disability in individual patients and can ultimately guide treatment choices. We identified 127 well-described subjects from four prior GEPCI studies that have at least one brain MRI with GEPCI data acquired at 3.0 Tesla. All 127 will be recruited to a single follow-up visit, in which each subject will undergo testing by a blinded examiner, with Expanded Disability Status Scale (EDSS), MS Functional Composite (MSFC) and Symbol Digit Modality Test (SDMT) and Montreal Cognitive Assessment (MoCA). MRI images will be re-analyzed for R2t* and Quantitative susceptibility maps (QSM). We will calculate average R2t* values from cortical and deep gray matter (GM), normal-appearing white matter and lesions, and determine individual regional patterns of CNS damage. We will also derive QSM from GEPCI to evaluate lesions and deep GM degeneration. In stored serum samples, we will measure serum NfL by single molecule array (Simoa). Associations of MRI changes and NfL levels with rate of disability accumulation will be determined. We all also build a multivariate model that best predicts future disability.

2. **KEYWORDS:** *Provide a brief list of keywords (limit to 20 words).*

Multiple Sclerosis, Biomarkers, Gradient Echo MRI, Neurofilament light chain, disability, prediction

3. **ACCOMPLISHMENTS:**

What were the major goals of the project?

The major goals of the project were as follows:

Major Task 1: Preparation to begin the study (100% completion of this goal was met on 5/14/2020)

a) Prepare IRB and HRPO submission, including informed consent, completed 10/26/2019.
b) Finalize consent form and human subjects protocol, completed 5/14/2019
c) Prepare Data entry capture forms in REDCAP, completed 5/14/2020
d) Train investigators and research coordinator in REDCAP data entry, completed 5/14/2020
e) Milestone: Completion of IRB and HRPO review and consent process. Completion of RedCap data elements. Milestone met 5/14/2020

Major Task 2: Follow-up visits

a) Subtask 1: Ensure that clinical investigators are trained in Expanded Disability Status Scale (EDSS). 100% completed 10/26/2019
b) Subtask 2: Ensure that research coordinator is trained in administration of Multiple Sclerosis Functional Composite (MSFC), Symbol Digit Modalities Test (SDMT). 100% completed 10/26/2019
c) Subtask 3: Contact and evaluate study subjects (n=127, relapsing remitting MS (RRMS)=60, secondary progressive MS (SPMS)=43 and primary progressive MS (PPMS)=24). Subtask is at 80% completion
d) Milestone: Follow-up visits completed (n=127). 75% completed

Major Task 3: Image analysis

a) Download GEPCI data and prepare for analysis (n=127), one scan per MS patient. 100% completed
b) Apply new processing to older GEPCI images (to obtain updated R2t* values), perform quality control on old and new images, 100% completed on 9/30/2021
c) Re-analysis of GEPCI images to obtain R2t* values in all brain areas. 100% completed
d) Generating GEPCI-Barcode areas and values. 30% completed
e) Generating Quantitative Susceptibility maps (QSM) data. 0% completed
f) Milestone: Completion of MRI re-analysis. 70% completed

Major Task 4: Serum analysis

a) Subtask 1: Purchase NfL assay, prepare for Single molecule array (Simoa) analysis. 0% completed.
b) Subtask 2: Coordinate timing of equipment use with Holtzman laboratory and ensure NfL assay is working accurately. 0% completed
c) Subtask 3: Analysis of samples from study subjects (n=88). One serum sample for each patient for a total of 88 samples. 0% completed
d) Milestone: Completion of serum analysis (n=88). 0% completed

Major Task 5: Data organization and analysis

a) Subtask 1: Obtain and organize previously collected data on study subjects (and enter in REDCAP). 75% completed
b) Subtask 2: Descriptive and summary statistics. 0% completed
c) Subtask 3: Study the association between MRI variables and future disability. 0% completed
d) Subtask 4: Study the association between serum NfL levels and future disability. 0% completed
e) Milestone: Complete initial data analysis. 0% completed

Major Task 6: Develop and test a multivariate model for the prediction of disability in MS.

a) Subtask 1: Construct a multivariate linear model for disability prediction (that incorporates MRI, serum makers and other important disease and patient variables). 0% completed
b) Subtask 2: Validate the multivariate model using a cross-validation technique. 0% completed
c) Subtask 3: Review study results, statistical analysis output and discuss results with study team. 0% completed
d) Milestone: Complete data analysis and study. 0% completed

What was accomplished under these goals?

For the first Major Task, 100% completion of this goal was achieved 5/14/2020. Under these goal initial materials, including the finalized consent form, were submitted to the Washington University IRB on 5/14/2019 and received approval on 10/26/2019. Data capture forms were developed for the project REDCAP database, which was moved from development into production on 5/14/2020. Investigators and study coordinators were trained on the entry of study data into the REDCAP database.

For the second major task, the training of clinical investigators and coordinators for the administration of their allocation study assessments was confirmed to be complete when IRB approval was received for this project. Participants with the oldest GEPCI MRIs were prioritized when participant contact began. For patients who are unable to be seen for a study visit in conjunction with a clinical appointment, with their permission via phone consent the chart review process is started with plans to complete an in-person study visit later. As of 10/15/2022, study coordinators have contacted 120 patients about the study; out of the 120 patients that have been contacted, 60 participants have completed an in-person study visit, 10 participants have completed phone interviews and 26 chart reviews visits have been completed under the consent waiver and 4 participants have upcoming visits (2 in-person, 2 phone) scheduled over the next two months. Elias Helal, a research analyst trained in chart review, has started reviewing the charts of consented subjects (or, when applicable, under waiver of consent).

For the third major task, MRI images for all 127 study participants have been searched for, located and downloaded (if available). New processing was applied to older GEPCI images to obtain updated R2t* values. Quality control was applied to both old and new images. For some of the older GEPCI images (from the CombiRx study), additional anatomical images were located to aid in the processing and segmentation of the GEPCI data. Out of 127 subject/scans, 16 cannot be included in this study (due to not having any usable GEPCI images or good quality anatomical images or due to significant artifact). Thus 111 images were re-processed (if needed) and made ready for analysis. For subjects with more than one GEPCI MRI, the oldest scan with good quality images was used (to allow a larger follow-up time).
Available scans from all subjects have now been analyzed. Those working with the imaging data are blinded to the clinical outcomes of the subjects. Reconstructed GEPCI scans were imported into MATLAB for further processing. We used an in-house MATLAB script developed by the Dr. Yablonskiy group to carry out further processing. For processing, first a Hanning filter was applied to correct for image artifacts. Then using a FSL Brain extraction tool we stripped the skull and extracted the brain from the images. Calculations for frequency mapping were run, then frequency unwrapping and frequency extension were performed. To complete processing, the first BOLD procedure calculation was completed. After completing the processing pipeline, we obtain the primary GPCI images, including 't1w', 'R2t', 'fre', 'dfre', 'Kesai', 'Ksi'. The resulting R2t maps were converted to NIFTI format and visually inspected for image artifacts.

We have adjusted our plan to run the serum analysis to now occur in the early part of 2023 and are in the process of purchasing the assays and run the testing.

For the fifth major task, data is being collected and organized through study review and chart review for input into the Redcap database.

The sixth major task is dependent on the completion of the previous five tasks and is not yet started.

What opportunities for training and professional development has the project provided?

Nothing to Report.

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?

In the next reporting period, we plan to finish contacting participants and conducting study visits. These are participants we expected to have difficulty reaching by phone, if we are unable to make contact with these patients we will complete a chart review under the waiver of consent. We will continue the chart review process, consolidating and validating data from subjects, previously collected study data and chart review into the current Redcap database. Chart review will proceed for subjects who have consented for the study (i.e. before or during the study visit), those who are unable to attend a study visit or those who cannot be reached (as detailed in our IRB application).
Almost all of the imaging analysis has now been completed and is awaiting clinical data from the study visits and chart review to commence the statistical analysis. Note that barcode generation will occur during the statistical analysis. Serum analysis will occur in the winter/spring of 2023, we have an order for the assays ready to go in January 2023.

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

While University-wide restrictions on in-person visits and participant hesitancy to come in for a research study visit during the ongoing pandemic has decreased, most of the later participants have preferred to complete in-person study visits when they are already on site for clinical appointments. As described in our previous annual reports, we are performing chart reviews ahead of scheduled in-person visits.

Actual or anticipated problems or delays and actions or plans to resolve them

The remaining participants to be contacted are those we expected to have difficulty reaching by phone, if we are unable to make contact with these patients we will complete a chart review under the waiver of consent. We modified our chart review procedures to be able to collect data prior to the completion of an in-person study visit and prioritizing participants who would likely only complete assessments by phone due to mobility or disease related limitations. For patients who scheduled for in-person clinic visit, we offering to complete their study visit on the same day to minimize the need for additional trips to our site.

Changes that had a significant impact on expenditures

Nothing to Report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Nothing to Report.

Significant changes in use or care of human subjects

Nothing to Report.

Significant changes in use or care of vertebrate animals

Nothing to Report.

Significant changes in use of biohazards and/or select agents

Nothing to Report.

6. PRODUCTS:

Publications, conference papers, and presentations

Nothing to Report.

Journal publications.

Nothing to Report.

Books or other non-periodical, one-time publications.

Nothing to Report.

Other publications, conference papers and presentations.

Nothing to Report.

Website(s) or other Internet site(s)

Nothing to Report.

Technologies or techniques

Nothing to Report.

Inventions, patent applications, and/or licenses

Nothing to Report.

Other Products

Nothing to Report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

Name	Dorothy Anne Cross
Project Role	Principal Investigator
Nearest person month worked	0.96
Contribution to Project	Dr. Cross has overseen the overall study, the build of the REDCap database, and performed study visits.
Funding Support	(effort supported by this grant)

Name	Dmitriy Yablonskiy
Project Role	Co-Investigator
Nearest person month worked	1.08

Contribution to Project	Dr. Yablonskiy has managed the MRI analysis and image quality control.
Funding Support	(effort supported by this grant)

Name	Biao Xiang
Project Role	Co-Investigator
Nearest person month worked	2
Contribution to Project	Dr. Xiang has contributed the analysis and procurement of MRI images for the study.
Funding Support	Effort supported from this grant through 6/30/2020, supported 100% from the National Multiple Sclerosis (MS) Society **beginning 7/1/2020**.

Name	Sayan Kahali
Project Role	Co-Investigator
Nearest person month worked	4
Contribution to Project	Dr. Kahali has contributed the analysis and procurement of MRI images for the study.
Funding Support	Work (effort) on this project supported by this grant (Dr. Kahali started on 7/1/2020 – **efforts ended 01/30/2022**)

Name	Salim Chahin
Project Role	Co-Investigator
Nearest person month worked	4.8
Contribution to Project	Dr. Chahin has overseen the overall study and the build of the REDCap database. He has performed study visits and trained study team members on data extraction during chart review, REDCap data entry and the cognitive and functional assessments for this study.
Funding Support	(effort supported by this grant)

Name	Matthew Brier
Project Role	MRI Analyst
Nearest person month worked	1
Contribution to Project	Dr. Brier is assisting with imaging analysis
Funding Support	Effort is supported by other grants

Name	Courtney Dula
Project Role	Research Coordinator
Nearest person month worked	2.4
Contribution to Project	Mrs. Dula has organized the subjects list, organized additional patient data, contacted study subjects, performed the study visits and help with the quarterly and annual reports.
Funding Support	(effort supported by this grant)

Name	Yassin Mryeoud
Project Role	Research Analyst
Nearest person month worked	0.2
Contribution to Project	Mr. Mryeoud is completing the chart review for enrolled study participants. **Ended December 2021**
Funding Support	Effort is supported by Washington University Department of Neurology

Name	Elias Helal

Project Role	Research Analyst
Nearest person month worked	0.2
Contribution to Project	Mr. Helal is completing the chart review for enrolled study participants.
Funding Support	(effort supported by this grant) **beginning October 2022**

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

See below and see attached for updated support documents.

Anne Cross (Principal Investigator)

Project(s) Ended:

Nothing to Report

Project(s) Started:

"T cell-intrinsic roles for the ZFP36 family proteins in MS and EAE" National Multiple Sclerosis Society. RG-211-38724. **Started 4/1/2022**

Dmitriy Yablonskiy (Co-Investigator)

Project(s) Ended:

Nothing to Report

Project(s) Started:

"Using Quantitative Gradient Echo MRI to Distinguish MOG Antibody Disorder from Multiple Sclerosis" National Institutes of Health. R03 NS121960. **Started 04/01/2021**

"In vivo Identification of Pre-Atrophic Brain Neurodegeneration in Prodromal Alzheimer Disease with Quantitative Gradient Recalled Echo MRI" National Institutes of Health. 1 RF1 AG077658-01. **Started 06/01/2022**

Salim Chahin (Co-Investigator)

Project(s) Ended:

"Cerebrospinal fluid-biomarkers-based diagnostic and prognostic models for Multiple Sclerosis" National Multiple Sclerosis Society. **Ended 6/30/2022**

Project(s) Started:

"Central Vein Sign: A Diagnostic Biomarker in Multiple Sclerosis (CAVS-MS)" National Institutes of Health. **Started 12/1/2021**

"Partners in virtual local delivery of specialized MS care based on location intelligence" Bristol Myers Squibb Foundation. **Started October 2022**

"Interventional, randomized, double-blind, placebo-controlled, parallel-group, phase 1b study investigating the effects of Lu AG06466 for the treatment of spasticity in patients with multiple sclerosis" Lundbeck. 19366A. **Started January 2022**

"Establishing Demographic and Comorbidity Specific Reference Ranges in Healthy Controls to Assess the Value of CNS Biomarkers in Monitoring Treatment Efficacy in Multiple Sclerosis

and Other Neurodegenerative Disorders – A Collaborative Approach" Bristol-Myers Squibb. **Started September 2022**

Biao Xiang (Co-Investigator)

Project(s) Ended:

Nothing to Report

Project(s) Started:

Nothing to report

Sayan Kahali (Co-Investigator)

Project(s) Ended:

Nothing to report

Project(s) Started:

Nothing to report

What other organizations were involved as partners?

Nothing to Report.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

Nothing to Report.

QUAD CHARTS:

Nothing to Report.

9. APPENDICES: *Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.*