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PRINCIPAL INVESTIGATOR: Tanuja Chitnis

CONTRACTING ORGANIZATION: Brigham and Women's Hospital

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6. AUTHOR(S)
Tanuja Chitnis

E-Mail: tchitnis@rics.bwh.harvard.edu

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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Brigham and Women's Hospital,
Inc., The
75 FRANCIS ST
BOSTON MA 02115-6110

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14. ABSTRACT

During this period, we have evaluated proteomic biomarkers and their associations with relapses and/or new gadolinium enhancing lesions (GD+) and 10-year outcomes in MS patients. We have specifically identified that serum neurofilament light chain (sNFL) is elevated within a 3-month window of new GD+ MRI lesions, which informs the frequency at which this biomarker may be surveyed in the clinical setting. We have also found that at a relapse or GD+ lesion that sNFL decreases with increasing age, with the opposite in remission samples. We have screened over 1100 other proteins and have identified a multivariate proteomic signature that more strongly associates with GD+ lesions than serum NFL alone. We have found that serum NFL levels within the first 3-5 years after disease onset strongly correlate with 10-year brain atrophy and deep grey matter volumes including thalamic volumes. These findings can inform the uses of sNFL and a multivariate proteomic biomarker in clinical MS settings.

15. SUBJECT TERMS
multiple sclerosis, longitudinal, predictive, biomarker, proteomic, metabolomic, lipid, neurofilament, relapse, MRI, disability, treatment, microRNA, antibody, biosamples, responder, inflammation, innate, adaptive immunity, neurodegeneration

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1. **INTRODUCTION:** The goal of this study is to evaluate proteomic, metabolomic and lipidomic biomarkers in the prediction of disease course in multiple sclerosis patients using data and biosamples from the Comprehensive Longitudinal Investigations in MS at the Brigham (CLIMB) Study. We have selected promising biomarkers identified from preliminary studies representing adaptive immunity, innate immunity and neurodegeneration. These will be evaluated in well characterized cohorts identified from the CLIMB study. Our intent is then to develop multivariable predictive models of disease course including relapses, disability and radiological outcomes.

2. **KEYWORDS:** multiple sclerosis, longitudinal, predictive, biomarker, proteomic, metabolomic, lipid, neurofilament, relapse, MRI, disability, treatment, microRNA, antibody, biosamples, responder, inflammation, innate, adaptive immunity, neurodegeneration.

3. **ACCOMPLISHMENTS:**
   a) **What were the major goals of the project?**
   Aim 1: Development of biomarkers for MS staging
   Aim 2: Development of predictive algorithms for short and long-term outcomes
   Aim 3: Development of composite panels and predictive models

   There were no significant changes to this project.
   The specific Aims during the first 24 months, along with their completion dates are listed below:

Specific Aims	Month	Site 1 – responsible key personnel
Aim 1: Development of biomarkers for MS staging	12	
a) Local IRB/IACUC Approval (all aims)	0-1	Dr. Chitnis
b) Preparation of datasets for nested cohorts	1-6	Dr. Chitnis
c) MRI validation – T1Gd+ lesions, T2 lesion accrual	3-9	Dr. Bakshi
d) Biomarker panel conduct	6-12	Dr. Chitnis + Dr. Weiner
e) Statistical analysis	12-15	Dr. Healy + Patsopoulos
f) Milestone(s) Achieved – reporting final results Aim 1	15	Dr. Chitnis
Aim 2: Development of predictive algorithms for short and long-term outcomes		
a) Preparation of datasets for nested cohorts	9-15	Dr. Chitnis
b) MRI analysis for T2LV, atrophy measures	12-26	Dr. Bakshi
c) Biomarker panel conduct	12-26	Dr. Chitnis + Dr. Weiner
d) Statistical analysis	26-28	Dr. Healy + Patsopoulos
e) Milestone(s) Achieved: Final results Aim 2	28	Dr. Chitnis

   b) **Major activities:** The major activities during the first 24 months of this project are as follows –
   AIM 1:
   - Local IRB approval – completed
   - Preparation of datasets – completed
   - MRI validation – T1Gd+ lesions, T2 lesion accrual – completed
   - Biomarker panel conduct – initiated and will be completed by month 26 of project. 9 manuscripts published or submitted (manuscripts 1-9 listed below); additional manuscripts are in preparation
   - Statistical analysis – conducted for abstracts/manuscripts below

   AIM 2:

- Preparation for datasets for nested cohorts: We have prepared datasets for large ongoing analyses on the following topics, and final analysis of biomarkers is ongoing:
    - Biomarkers of high vs. low efficacy early treatment
    - Biomarkers of secondary progressive MS
    - Biomarkers of treatment response to ocrelizumab
    - Biomarkers of treatment response to teriflunomide
    - Biomarkers of treatment response to fingolimod
    - Biomarkers and predictors of 10-year outcomes in MS
- MRI analysis for T2LV and brain atrophy is completed for the datasets described above in (a). These values will be incorporated into the final analysis.
- Biomarker panel and statistical analysis for datasets described in (a) is ongoing.
- We have also begun the assembly of composite datasets which include multivariate predictors and machine-learning models (see items 10-11 below).

c) Specific objectives: Our specific objectives for the first 24 months are as follows:

- IRB approval and preparation of datasets.
- We have completed MRI validation in nested cohorts for this study.
- We have completed conduct of biomarker panels including studies of serum neurofilament light chain and inflammation-associated proteomics.
- Additional biomarker analyses including miRNA, Ag array panels, astrocytic and neuronal biomarkers, cytokine panel and lipid biomarkers is ongoing for cohorts above.

d) Significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative): We have completed the following studies which have been submitted for publication and/or major congresses (see detailed citations in section 6):

1) Neurofilament light chain serum levels correlate with 10-year MRI outcomes in multiple sclerosis.
    - Major findings: This study found in 125 MS patients with annual serum sampling that averaged serum NFL levels drawn during the first 3-5 years of MS disease onset correlated with brain atrophy at 10 years.
    - This work was published in October 2018.
    - This work was also presented at the 2019 ACTRIMS meeting in Dallas, TX.

2) Temporal association of sNfL and gad-enhancing lesions in multiple sclerosis.
    - Major findings: This study of 94 MS patients found that sNFL was elevated 90 days pre and post-gadolinium enhancing lesions; which may inform its use in clinical practice. sNFL was note significantly elevated at a relapse without Gd+ lesions. We also found that sNFL was elevated in spinal cord lesions and brainstem lesions compared to other lesion locations.
    - This work was published in June 2020.
    - This work was presented at the 2019 ECTRIMS Congress in Stockholm, Sweden (platform presentation: Serum neurofilament light chain levels are increased within three months of new gadolinium enhancing lesions in multiple sclerosis)

3) Differential association of Age and Serum neurofilament light chain in remission and after Gd+ lesions in MS.
    - Major findings: sNFL levels during remission (non-relapse, non-Gad) sample showed an increasing association with patient age) adjusted estimate=1.2% yearly increase, 95% CI=0.3%-2.0%, p=0.008). However, sNFL levels taken within 90 days prior to a Gd+ lesion showed no age-associated increase. Further, we report a negative interaction between age and gadolinium-enhancing lesion status (adjusted estimate=1.7% annual decrease, 95% CI= -2.9% – -0.6%, p=0.003).
    - This work was submitted to a journal for publication in August 2020
    - This work was presented as a poster at the ACTRIMS 2020 meeting, West Palm Beach, FL, 2020.

4) Serum NFL levels in first five years correlate with 10-year Deep Gray Matter volumes in multiple sclerosis.

- Major findings: A negative association was seen between averaged annual NFL and 10-year GMF values, which included years 1-5 and 1-6 values (unadjusted $p<0.05$; adjusted analysis $p<0.05$). The 10-year WMF analysis showed a similar negative association for all averaged year (1-10) combinations (unadjusted $p<0.05$; adjusted analysis $p<0.05$). Negative associations were also seen for the 10-year thalamus analysis and all averaged year (1-10) NFL combinations (unadjusted $p<0.05$; adjusted analysis $p<0.05$). Similar negative associations were also seen for both the 10-year Caudate and Globus Pallidas analysis with averaged NFL values for years 1-5, 1-6, 1-7 and 1-10 respectively (unadjusted $p<0.05$; adjusted analysis $p<0.05$).
- This work was submitted to a journal for publication in August 2020.
- This work was presented as a poster at the ACTRIMS 2020 meeting, West Palm Beach, FL, 2020.

5) Classification of High Versus Low Annualized Relapse Rate Status in Subjects with Relapsing-Remitting Multiple Sclerosis Using Multivariate Serum Protein Biomarker Models.
   - Major findings: This study evaluated over 1000 proteomic markers in in RRMS patients with high (≥1 relapses/year) and low (≤0.2 relapses/year) relapse rates, and found that sNFL as well as several other inflammatory markers were associated with a higher relapse rate (CD6, IL-1RT2, COL4A1, LEPR, BSCAN).
   - This work was presented as a poster presentation, 2019 ECTRIMS Congress, Stockholm, Sweden.

6) Multivariate Protein Biomarker Models More Accurately Predict Multiple Sclerosis MRI Disease Activity Compared to Serum Levels of Neurofilament Light Chain Alone.
   - Major findings: Over 1000 proteomic biomarkers were evaluated in 326 patients including 226 paired samples in patients with a Gd+ and Gd- state. We found that a multivariate classifier that consisted of 10 biomarkers (including sNfL) improved the classification of Gd+ and Gd- samples with 0.896 ± 0.046 Accuracy and 0.959 ± 0.023 AUC, compared to an AUC of 0.686 with sNFL alone. Furthermore, sNfL alone was unable to distinguish samples with 0 vs. 1 lesion ($p = 0.138$) while multivariate biomarker models were able to at a statistically significant level ($p < 0.01$).
   - This work was presented at the 2019 ECTRIMS Congress, Stockholm, Sweden.
   - This work was presented at the 2020 ACTRIMS Forum, West Palm Beach, FL, US.

7) Development of a Custom Multivariate Proteomic Serum Based Assay for Association with Radiographic and Clinical Endpoints in MS.
   - Major findings: A 21 protein biomarker panel was developed from the work descried in F and validated in two cohorts, the CLIMB study from the Brigham and Women's Hospital and the EPIC study from the University of California, San Francisco. Multivariate statistical ensembles restricted to the expression levels of the biomarkers selected for thecustom assay achieved AUC performance of 0.827 for classification of the presence of Gd+ lesions, 0.802 for classification of clinically defined relapse status, and 0.930 for the classification of patients with Low ARR (≤0.2 relapses) vs High ARR (≥1.0 relapses). A multivariate model utilizing shifts in biomarker expression in longitudinally paired samples achieved the highest observed performance of 0.950 for classification of Gd+ lesion presence. In each case, the multivariate models significantly outperformed (p-value <0.05) the AUC of the highest performing univariate biomarker.
   - This work was presented as a poster presentation, 2020 MSVirtual meeting (combined ACTRIMS/ECTRIMS meeting).

8) Association of Serum Neurofilament Biomarker Levels with Quality of Life and Healthcare Utilization in Patients with Multiple Sclerosis
   - Major findings: A total of 304 MS patients with a mean age of 32.9 years, average EDSS of 1.6 (1.5) and baseline sNFL of 8.8 (range 1.23-78.3) pg/ml were enrolled in the cohort. Overall, baseline sNFL correlated with baseline MSQOL physical composite ($p=0.035$) and baseline EDSS ($p=0.002$). Other PRO measures at baseline did not show a significant relationship with baseline sNFL. Average of baseline and follow-up sNfL correlated with MSQOL physical-role limitations ($p=0.043$) and social-functioning ($p=0.034$) at 24-month follow-up. We found a trend for numerically higher sNfL levels in non-persistent patients compared to those who were persistent to treatment (11.13 vs 8.53 pg/ml, $p=0.093$) measured as average of baseline

and 24-month values. Baseline NfL was associated number of intravenous steroid infusions (p=0.013) while average of baseline and 12 months NfL values was related to inpatient stays at 12-months (p=0.053).
- This work has been submitted for publication in September 2020.
- This work was presented at the American Academy of Neurology Virtual meeting, 2020.

9) Obesity is associated with Optic Neuritis severity in male patients with Multiple Sclerosis.
   - Major findings: We included 61 patients with clinically isolated syndrome (CIS) and MS who had a history of AON and were enrolled in the Comprehensive Longitudinal Investigation at the Brigham and Women's Hospital (CLIMB). We recorded AON severity and recovery according to visual acuity outcomes recorded before, at, and, after the relapse. We measured the serum concentration of estradiol, leptin, testosterone, sex hormone-binding globulin, and vitamin D. Results: Male patients with moderate/severe AON had significantly higher BMI than male patients with mild AON (31.26 kg/m^2 vs 25.73 $kg/m^{2,}$ p = 0.03). Males with moderate/severe AON had higher serum estradiol levels than males with mild AON (32.24 nmol/L vs 23.06 nmol/L, p=0.04). Male patients also showed a near-significant association between serum leptin and AON severity (moderate/severe AON: 12.29 ng/mL vs mild AON: 4.1 ng/mL, p = 0.06). These observations were not replicated in female patients. We failed to find any association between AON recovery and BMI, as well as between serum hormones and AON recovery.
   - This work was submitted for publication in September 2020.
   - This work was presented at the American Academy of Neurology Virtual meeting, 2020.

10) Determination of early prognostic factors associated with 10-year outcome in multiple sclerosis: a comprehensive single centre study.
   - Major findings: One-hundred-twenty-two patients with 10 years of complete data were included. Thirteen (11%) maintained NEDA beyond year 3, and 52 (43%) escalated to HET. Eighty-six (70.5%) had 239 attacks beyond year 3, more likely to be younger (OR 0.93, p=0.009) with higher baseline T2 lesion volume. MRI lesion formation was associated with BMI (p=0.006), vascular comorbidities (p=0.006), NFL (p=0.027), and early MRI activity (p=0.015). Brain atrophy was associated with neurologic comorbidity (p=0.005) and NFL (p=0.008). EDSS progression was associated with injectables (p=0.018) and less likely in patients with monofocal sensory attacks (p=0.026). Final EDSS was associated with 3-year EDSS (p=0.001), injectables (p=0.017), and escalation to HET within 3 years of symptom onset (p=0.022).
   - This work was submitted to the American Academy of Neurology (AAN) 2021 Meeting.

11) Association of Early Prognostic Factors with 10-Year Brain Atrophy and Disability in Multiple Sclerosis: A Comprehensive Single Centre Study
   - Major findings: 452 patients were analyzed, with mean 34.8 years at first symptoms and 4.2% denoted as progressive at the first visit. At year 10, median EDSS was 1.5 (range 0-8.0), with 338 (74.8%) categorized as benign and 30 (6.6%) as aggressive. Compared with benign, aggressive MS had higher brain atrophy (p=.0008), T2 lesion volume (p=.0097), older age at first symptoms (33.5±9.4 vs. 43.7±11.1, p<.0001), positive smoking history (35% vs. 63%, p=.002), have higher EDSS at first visit (1 vs. 3.5, p<.0001), and within the first 3 years have poor recovery from attacks (29% vs. 93%, p<.0001) and motor attacks (25% vs. 63%, p<.0001). Patients with benign MS were more likely Caucasian (89% vs. 77%, p=.038) with optic neuritis or sensory attacks (30% vs. 13%, p=.048, 66% vs. 43%, p<.013, respectively), as well as have received steroids within the first 3 years (36% vs. 10%, p=.005). Sex, family history, vitamin D levels, BMI, vascular or neurologic comorbidities, as well as number of attacks in first 1 or 3 years did not significantly associated with 10-year EDSS. Brain atrophy was worse in patients who were older at first symptoms (p<.0001), male (p=.021), and had history of smoking (p=.005). On multivariable analysis first EDSS, progressive disease, and attack history was not significantly associated with 10-year brain atrophy.
   - This work was submitted to the ACTRIMS 2021 Forum.

e) Other achievements: The following other achievements related to this project include meeting reports and reviews, led by Dr. Chitnis around the theme of this research grant.

- Dr. Chitnis co-chaired the 2019 ACTRIMS Forum meeting with a theme of "Precision Medicine for MS" with over 1200 attendees (Dallas, TX), which featured talks and presentations on precision biomarkers for MS diagnosis and prognosis.
- Dr. Chitnis co-edited a special edition of Multiple Sclerosis Journal (in preparation) which features 12 articles on the theme of Precision Medicine in MS, with coauthors from the ACTRIMS Forum meeting 2019, and featuring articles on biomarkers and predictive algorithms for MS. This include an overview article entitled "A Roadmap to Precision Medicine for MS" authored by T. Chitnis and A. Prat.
- Invited editorial: "Serum NFL levels should be used to monitor multiple sclerosis evolution" by Dr. Chitnis, accepted for publication in Multiple Sclerosis Journal (September 2019).

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

- Professional Development:
  - Mattia Rosso, MD, PhD – additional training in biomarker analysis, statistical analysis
  - Neda Sattarnezhad, MD – additional training in biomarker analysis, statistical analysis
  - Hajime Yano MD – additional training in biomarker analysis, statistical analysis
  - Marinos Sotiropoulos MD – additional training in biomarker analysis, statistical analysis
  - Kristin Galetta, MD – additional training in biomarker analysis, statistical analysis
  - Duong Chu (pre-MD) – additional training in biomarker analysis, statistical analysis
  - Gauruv Bose, MD – additional training in biomarker analysis, statistical analysis
  - Vanessa Moreira Ferreira, MD – additional training in biomarker analysis, statistical analysis
- Opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project:
  - Mattia Rosso, MD PhD – one-on-one work with a mentor; one-on-one work with biostatistician and development of statistical analysis skills; attendance of workshops on biomarker analysis, network medicine and biostatistics; attendance and presentation of abstracts at international conference (ECTRIMS 2019).
  - Neda Sattarnezhad, MD – one-on-one work with a mentor; attendance and presentation of abstracts at international conference (ECTRIMS 2019).
  - Hrishikesh Lokhande, MSc – attendance of workshops on bioinformatics tools, one-on-one work with biostatistician and presentation of abstracts.
  - Kristin Galetta, MD - one-on-one work with a mentor; attendance and presentation of abstracts at national conference (AAN 2020).
  - Duong Chu (pre-MD) - one-on-one work with a mentor; attendance and presentation of abstracts at national conference (AAN 2020).
  - Gauruv Bose, MD – one-on-one work with a mentor; attendance and submission of abstracts at national conference (AAN 2021, ACTRIMS 2021)
  - Vanessa Moreira Ferreira, MD – one-on-one work with a mentor; attendance and preparation of manuscripts in process.

g) How were the results disseminated to communities of interest?

- Publications: 2 published; 6 additional publications submitted.
- Abstract presentations at major meetings: 9 presented.

h) What do you plan to do during the next reporting period to accomplish the goals?

1) We plan to continue biomarker analysis in the identified cohorts. In the upcoming year, we will focus on metabolomic and miRNA analysis in the nested cohorts.
2) We will begin to develop composite panels and predictive models.
3) In order to accomplish these goals, we will continue to analyze biosamples for the identified biomarkers and continue to conduct statistical analysis for the identified outcome measures.

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**
  - No changes
- **Actual or anticipated problems or delays and actions or plans to resolve them**
  - No problems
- **Changes that had a significant impact on expenditures**
  - No changes that had a significant impact on expenditures
- **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**
  - No changes
- **Significant changes in use or care of human subjects**
  - No changes
- **Significant changes in use or care of vertebrate animals.**
  - NA
- **Significant changes in use of biohazards and/or select agents**
  - NA

6) PRODUCTS:

a) Publications, conference papers, and presentations

1) Major findings: This study found in 125 MS patients with annual serum sampling that averaged serum NFL levels drawn during the first 3-5 years of MS disease onset correlated with brain atrophy at 10 years.
   a. Neurofilament light chain serum levels correlate with 10-year MRI outcomes in multiple sclerosis. Chitnis T, Gonzalez C, Healy BC, Saxena S, Rosso M, Barro C, Michalak Z, Paul A, Kivisakk P, Diaz-Cruz C, Sattarnezhad N, Pierre IV, Glanz BI, Tomic D, Kropshofer H, Häring D, Leppert D, Kappos L, Bakshi R, Weiner HL, Kuhle J. Ann Clin Transl Neurol. 2018 Oct 16;5(12):1478-1491. doi: 10.1002/acn3.638. eCollection 2018 Dec. PMID: 30564615; PMCID: PMC6292183.
      - This work was published in October 2018.
      - This work was also presented at the 2019 ACTRIMS meeting in Dallas, TX.
      - Acknowledgement of federal support – yes

2) Major findings: sNfL levels during remission (non-relapse, non-Gad) sample showed an increasing association with patient age) adjusted estimate=1.2% yearly increase, 95% CI=0.3%-2.0%, p=0.008). However, sNFL levels taken within 90 days prior to a Gd+ lesion showed no age-associated increase. Further, we report a negative interaction between age and gadolinium-enhancing lesion status (adjusted estimate=1.7% annual decrease, 95% CI= -2.9% – -0.6%, p=0.003).
   a. Temporal association of sNfL and gad-enhancing lesions in multiple sclerosis. Rosso M, Gonzalez CT, Healy BC, Saxena S, Paul A, Bjornevik K, Kuhle J, Benkert P, Leppert D, Guttmann C, Bakshi R, Weiner HL, Chitnis T. Ann Clin Transl Neurol. 2020 Jun;7(6):945-955. doi: 10.1002/acn3.51060. Epub 2020 May 25. PMID: 32452160; PMCID: PMC7318095.
      - This work was published in June 2020.
      - Acknowledgement of federal support – yes

b. Serum neurofilament light chain levels are increased within three months of new gadolinium enhancing lesions in multiple sclerosis. Rosso, M. (2019)
   - This work was presented at the 2019 ECTRIMS meeting in Stockholm, Sweden.
   - Acknowledgement of federal support – yes

3) Major findings: sNFL levels during remission (non-relapse, non-Gad) sample showed an increasing association with patient age) adjusted estimate=1.2% yearly increase, 95% CI=0.3%-2.0%, p=0.008). However, sNFL levels taken within 90 days prior to a Gd+ lesion showed no age-associated increase. Further, we report a negative interaction between age and gadolinium-enhancing lesion status (adjusted estimate=1.7% annual decrease, 95% CI= -2.9% – -0.6%, p=0.003).
   a. Differential association of Age and Serum neurofilament light chain in remission and after Gd+ lesions. Rosso M, Healy BC, Saxena S, Paul A, Bjornevik K, Kuhle J, Benkert P, Leppert D, Guttmann C, Bakshi R, Weiner HL, Chitnis T.
      - This work was submitted to a journal for publication in August 2020.
      - Acknowledgement of federal support – yes
   b. Age Impacts the Association Between Gd+ Lesions and Serum Neurofilament Light in Patients with Multiple Sclerosis. Rosso M, Healy BC, Saxena S, Paul A, Bjornevik K, Kuhle J, Benkert P, Leppert D, Guttmann C, Bakshi R, Weiner HL, Chitnis T.
      - This work was presented as a poster at the ACTRIMS 2020 meeting, West Palm Beach, FL, 2020.
      - Acknowledgement of federal support – yes
      - *We have included this poster in the appendices.*

4) Major findings: A negative association was seen between averaged annual NFL and 10-year GMF values, which included years 1-5 and 1-6 values (unadjusted $p<0.05$; adjusted analysis $p<0.05$). The 10-year WMF analysis showed a similar negative association for all averaged year (1-10) combinations (unadjusted $p<0.05$; adjusted analysis $p<0.05$). Negative associations were also seen for the 10-year thalamus analysis and all averaged year (1-10) NFL combinations (unadjusted $p<0.05$; adjusted analysis $p<0.05$). Similar negative associations were also seen for both the 10-year Caudate and Globus Pallidas analysis with averaged NFL values for years 1-5, 1-6, 1-7 and 1-10 respectively (unadjusted $p<0.05$; adjusted analysis $p<0.05$).
   a. Serum NfL levels in first five years predict 10-year thalamic atrophy in patients with MS. Lokhande H, Rosso M, Tauhid S, Chu R, Healy BC, Saxena S, Barro C, Paul A, Polgar-Turcsanyi M, Anderson M, Glanz BI, Kropshofer H, Granziera C, Leppert D, Kappos L, Kuhle J, Weiner HL, Bakshi R, Chitnis T.
      - This work was submitted to a journal for publication in August 2020.
      - Acknowledgement of federal support – yes
   b. Serum NfL levels in the first five years correlate with 10-year deep gray matter MRI volumes in multiple sclerosis. Lokhande H, Tauhid S, Chu R, Rosso M, Healy BC, Saxena S, Diaz-Cruz C, Sattarnezhad N, Paul A, Glanz BI, Barro C, Kuhle J, Leppert D, Bakshi R, Weiner HL, Chitnis T. (2020)
      - This work was presented as a poster at the ACTRIMS 2020 meeting, West Palm Beach, FL, 2020.
      - Acknowledgement of federal support – yes
      - *We have included this poster in the appendices.*

5) Major findings: This study evaluated over 1000 proteomic markers in in RRMS patients with high (≥1 relapses/year) and low (≤0.2 relapses/year) relapse rates, and found that sNFL as well as several other inflammatory markers were associated with a higher relapse rate (CD6, IL-1RT2, COL4A1, LEPR, BSCAN).
   a. Classification of High Versus Low Annualized Relapse Rate Status in Subjects with Relapsing-Remitting Multiple Sclerosis Using Multivariate Serum Protein Biomarker Models. Sattarnezhad N, Saxena S, Gonzalez C, Lokhande H, Glanz, B, Qureshi F, Becich M, Osan R, Weiner H, Chitnis T.
      - This work was presented as a poster presentation, 2019 ECTRIMS Congress, Stockholm, Sweden.
      - Acknowledgement of federal support – yes

6) Major findings: Over 1000 proteomic biomarkers were evaluated in 326 patients including 226 paired samples in patients with a Gd+ and Gd- state. We found that a multivariate classifier that consisted of 10 biomarkers (including sNfL) improved the classification of Gd+ and Gd- samples with 0.896 ± 0.046 Accuracy and 0.959

± 0.023 AUC, compared to an AUC of 0.686 with sNFL alone. Furthermore, sNfL alone was unable to distinguish samples with 0 vs. 1 lesion ($p = 0.138$) while multivariate biomarker models were able to at a statistically significant level ($p < 0.01$).

a. Multivariate Protein Biomarker Models More Accurately Predict Multiple Sclerosis MRI Disease Activity Compared to Serum Levels of Neurofilament Light Chain Alone. Chitnis T, Yano H, Saxena S, Lokhande H, Sattarnezhad N, Manieri MC, Paul A, Saleh F, Collins M, Glanz B, Guttmann C, Bakshi R, Qureshi F, Becich M, Osan R, Gehman V, Weiner H.
   - This work was presented as a poster presentation, 2019 ECTRIMS Congress, Stockholm, Sweden.
   - This work was also presented as a poster presentation, 2020 ACTRIMS Forum, West Palm Beach, FL, US.
   - Acknowledgement of federal support – yes
   - *We have included this poster in the appendices.*

7) Major findings: A 21 protein biomarker panel was developed from the work descried in F and validated in two cohorts, the CLIMB study from the Brigham and Women's Hospital and the EPIC study from the University of California, San Francisco. Multivariate statistical ensembles restricted to the expression levels of the biomarkers selected for thecustom assay achieved AUC performance of 0.827 for classification of the presence of Gd+ lesions, 0.802 for classification of clinically defined relapse status, and 0.930 for the classification of patients with Low ARR (≤0.2 relapses) vs High ARR (≥1.0 relapses). A multivariate model utilizing shifts in biomarker expression in longitudinally paired samples achieved the highest observed performance of 0.950 for classification of Gd+ lesion presence. In each case, the multivariate models significantly outperformed (p-value <0.05) the AUC of the highest performing univariate biomarker.

   a. Development of a Custom Multivariate Proteomic Serum Based Assay for Associate with Radiographic and Clinical Endpoints in MS. Chitnis T, Becich M, Bove R, Cree B, Gehman V, Gomez R, Hauser SL, Henry R, Katrib A, Lokhande H, Oksenberg JR, Paul A, Qureshi F, Santaniello A, Sattarnezhad N, Saxena S, Weiner H, Wilson M, Yano H, Baranzini SE.
      - This work was presented as a poster presentation, 2020 MSVirtual, (combined ACTRIMS/ECTRIMS virtual meeting).
      - Acknowledgement of federal support – yes

8) Major findings: A total of 304 MS patients with a mean age of 32.9 years, average EDSS of 1.6 (1.5) and baseline sNFL of 8.8 (range 1.23-78.3) pg/ml were enrolled in the cohort. Overall, baseline sNFL correlated with baseline MSQOL physical composite ($p=0.035$) and baseline EDSS ($p=0.002$). Other PRO measures at baseline did not show a significant relationship with baseline sNFL. Average of baseline and follow-up sNfL correlated with MSQOL physical-role limitations ($p=0.043$) and social-functioning ($p=0.034$) at 24-month follow-up. We found a trend for numerically higher sNfL levels in non-persistent patients compared to those who were persistent to treatment (11.13 vs 8.53 pg/ml, $p=0.093$) measured as average of baseline and 24-month values. Baseline NfL was associated number of intravenous steroid infusions ($p=0.013$) while average of baseline and 12 months NfL values was related to inpatient stays at 12-months ($p=0.053$).

   a. Association of Serum Neurofilament Biomarker Levels with Quality of Life and Healthcare Utilization in Patients with Multiple Sclerosis. Galetta K, Deshpande C, Healy BC, Glanz B, Ziehn M, Saxena S, Paul A, Saleh F, Collins M, Gaitain-Walsh P, Castro-Mendoza P, Weiner HL, Chitnis T.
      - This work has been submitted for publication in September 2020.
      - This work was also presented at the 2020 American Academy of Neurology Annual Meeting (virtual).
      - Acknowledgement of federal support – yes

9) Major findings: We included 61 patients with clinically isolated syndrome (CIS) and MS who had a history of AON and were enrolled in the Comprehensive Longitudinal Investigation at the Brigham and Women's Hospital (CLIMB). We recorded AON severity and recovery according to visual acuity outcomes recorded before, at, and, after the relapse. We measured the serum concentration of estradiol, leptin, testosterone, sex hormone-binding globulin, and vitamin D. Results: Male patients with moderate/severe AON had significantly higher BMI than male patients with mild AON (31.26 kg/m^2 vs 25.73 $kg/m^{2,}$ $p = 0.03$). Males with moderate/severe AON had higher serum estradiol levels than males with mild AON (32.24 nmol/L vs 23.06 nmol/L, $p=0.04$). Male patients also showed a near-significant association between serum leptin and AON

severity (moderate/severe AON: 12.29 ng/mL vs mild AON: 4.1 ng/mL, p = 0.06). These observations were not replicated in female patients. We failed to find any association between AON recovery and BMI, as well as between serum hormones and AON recovery.

a. Obesity is associated with Optic Neuritis severity in Male patients with Multiple Sclerosis. Chu DT, Rosso M, Gonzalez CT, Saxena S, Healy BC, Weiner HL, Chitnis T.
   - This work has been submitted for publication in September 2020.
   - This work was also presented at the 2020 American Academy of Neurology Annual Meeting (virtual).
   - Acknowledgement of federal support – yes

- **Books or other non-periodical, one-time publications:** None.

b) Website(s) or other Internet site(s)

- www.climbstudy.org

c) Technologies or techniques

- Pending technology on Proteomic markers associated with a Gd+ lesion (in collaboration with Octave Biosciences).

d) Inventions, patent applications, and/or licenses

- None.

e) Other Products

- data or databases; This grant supports in part the CLIMB database infrastructure required for this project.
- biospecimen collections; This grant has supported the identification of nested cohorts of MS patients in the CLIMB study which can be used for future studies.
- audio or video products; None
- software; None
- models; Multivariate predictive models of Gd+ lesions were developed as a result of this project; Predictive models of 10-year outcomes were developed as a result of this project.
- educational aids or curricula; Abstracts and talks presented at major congresses (ACTRIMS 2019, ECTRIMS 2019, AAN 2020 and MSVirtual 2020) were developed as a result of this project.
- instruments or equipment; NA
- research material (e.g., Germplasm; cell lines, DNA probes, animal models); NA
- clinical interventions; NA
- new business creation; no
- other; NA

7) PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

a) What individuals have worked on the project?

Name:	Mark Anderson
Project Role:	MRI Analyst
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	2.60 CM
Contribution to Project:	Mr. Anderson is an MRI analyst and will conduct the processing of MRIs for volumetric analysis
Funding Support:	

Name:	Rohit Bakshi
Project Role:	Co-Investigator
Researcher Identifier (e.g. ORCID ID):	https://orcid.org/0000-0001-8601-5534

Nearest person month worked:	1.40 CM
Contribution to Project:	He will supervise the conduct of MRI analysis for this project including the derivation of brain volumetrics and lesion volumes. He will work with Dr. Chitnis on data analysis and manuscript preparation.
Funding Support:	See active support attached.

Name:	Tanuja Chitnis
Project Role:	PD/PI
Researcher Identifier (e.g. ORCID ID):	https://orcid.org/0000-0002-9897-4422
Nearest person month worked:	1.80 CM
Contribution to Project:	She will oversee all aspects of the project including the management of the dataset, subject identification and phenotyping, and biosample analysis. She will oversee data analysis and manuscript development. She will oversee the postdoctoral fellows, technicians and will coordinate meetings with the bioinformatician and collaborators. Dr. Chitnis will be primarily responsible for all study design and manuscript preparation
Funding Support:	See active support attached.

Name:	Brian Healy
Project Role:	Biostatistician
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	0.60 CM
Contribution to Project:	He will conduct the biostatistical analysis for the project in concert with Dr. Patsopoulos and the bioinformatician. He will participate in manuscript preparation.
Funding Support:	See active support attached.

Name:	Hrishikesh Lokhande
Project Role:	Bioinformatician
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	2.40 CM
Contribution to Project:	He will participate in bioinformatics analysis for this project. He will meet weekly with Dr. Chitnis and her team.
Funding Support:	

Name:	Anu Paul
Project Role:	Research Technician
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	2.40 CM
Contribution to Project:	She will assist in sample procurement, processing, and conduct of proteomic assays.
Funding Support:	

Name:	Mattia Rosso
Project Role:	Postdoc
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	4.00 CM
Contribution to Project:	He will participate in the conduct patient selection, data validation and data analysis. He will work with Dr. Chitnis and the team bioinformatics data analysis and manuscript preparation.

Funding Support:	

Name:	Shrishti Saxena
Project Role:	Laboratory Technician
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	4.50 CM
Contribution to Project:	She will participate in the conduct patient selection, data validation and data analysis. She will work with Dr. Chitnis and the team bioinformatics data analysis and manuscript preparation.
Funding Support:	

Name:	Howard Weiner
Project Role:	Co-Investigator
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	0.60 CM
Contribution to Project:	He will provide input on the biological assays and immune markers being assessed. He will work with Dr. Chitnis on development of manuscripts
Funding Support:	See active support attached.

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

- Please find attached the active other support for the PD/PI (Chitnis) and senior/key personnel (Weiner, Bakshi, Patsopoulos, Healy)

c) What other organizations were involved as partners?

- **Organization Name:** University of Basel
  - **Location of Organization:** Basel, Switzerland
  - **Partner's contribution to the projec**t conduct of biosample analysis
  - **Financial support -** N/A
  - **In-kind support** - N/A
  - **Facilities** – the University of Basel conducted biomarker analysis (serum NFL) using the SIMOA machine on a subset of patients from the CLIMB cohort.
  - **Collaboration** – Investigators from the University of Basel who participated in this biomarker analysis are included as coauthors in several manuscripts.
  - **Personnel exchanges** – N/A
  - **Other -** N/A
- **Organization Name:** Octave Biosciences
  - **Location of Organization:** Menlo Park, California
  - **Partner's contribution to the projec**t contribution to statistical analysis
  - **Financial support –** Provided funding for biomarker analysis
  - **In-kind support** - N/A
  - **Facilities** – provided biomarker analysis platform through OLINK (located in Boston, MA) for nested cohorts within the CLIMB Study.
  - **Collaboration** – Investigators from Octave Biosciences who participated in this biomarker analysis are included as coauthors on abstracts of these results.
  - **Personnel exchanges** – N/A
  - **Other** – N/A

8) SPECIAL REPORTING REQUIREMENTS:

- **COLLABORATIVE AWARDS:** NA

- **QUAD CHARTS:** NA

9) APPENDICES:

a) Key Personnel Active Research Support
   - Bakshi
   - Chitnis
   - Healy
   - Patsopoulos
   - Weiner

b) Selected Poster Presentations (noted in section 6)
   - Age Impacts The Association Between Gd+ Lesions And Serum Neurofilament Light In Patients With Multiple Sclerosis (Rosso, et al) – poster presentation, ACTRIMS 2020
   - Serum NFL levels in first five years correlate with 10-year Deep Gray Matter MRI volumes in multiple sclerosis (Lokhande, et al.) – poster presentation, ACTRIMS 2020
   - Multivariate Protein Biomarker Models Better Predict Multiple Sclerosis MRI Disease Activity Compared to Serum Levels of Neurofilament Light Chain Alone (Chitnis et al.) – poster presentation, ACTRIMS 2020

RESEARCH SUPPORT

BAKSHI, Rohit, M.D., M.A.

ACTIVE

Collaborative Research Agreement (PI Singhal) 06/23/20 – 06/22/25 0.12
Calendar Novartis Pharma AG (Cumulative Total) 1.0% Effort
Total Direct Costs)

Open-label, single-blinded, observational, prospective, 9-month study to assess the efficacy of Ofatumumab on microglia in patients with relapsing forms of multiple sclerosis

The *primary* objective of this study is the assessment of the effect of ofatumumab over 9 months on the evolution of microglia pathology in patients with relapsing MS. The *secondary* objectives include the following assessments over 9 months: (a) To determine the time course of effect of Ofatumumab on microglial activation and its relationship with peripheral B-cell depletion, serum neurofilament light chain and glial-fibrillary acid protein levels and other serum biomarkers (IP-10, ITAC, MCP-1 and MIP-3b). (b) To determine the relationship of PET changes following Ofatumumab initiation with 3T MRI changes and clinical parameters.

Aim 1: To determine the effect of Ofatumumab on microglial activation in MS over 9 months.

Aim 2: To determine the time course of effect of Ofatumumab on microglial activation and its relationship with peripheral B-cell depletion, serum neurofilament light (sNfL) chain and glial-fibrillary acid protein (GFAP) levels and other serum biomarkers (IP-10, ITAC, MCP-1 and MIP-3b).

Aim 3: To determine the relationship of PET changes following Ofatumumab initiation with 3T MRI changes and clinical parameters.

Role: Co-Investigator

Sponsor POC: Brandon Brown, brandon.brown@novartis.com, (617) 959-1510, 181 Massachusetts Ave, Cambridge, MA 02139

RPC1063-MS-13914 (PI Bakshi) 03/10/20 – 03/09/25 0.12 Calendar
Celgene Corporation (Cumulative Total) 1.0% Effort
(Total Direct Costs)

Cortical and meningeal involvement in multiple sclerosis: A 7T MRI study

This is an observational study following MS patients for one year to assess the relationship among cortical lesions, cortical atrophy, and meningeal enhancement using 7T MRI. We will also link these to physical disability and cognition.

Role: PI

Sponsor POC: David Berk, dberk@celgene.com, 908-679-7768, 200 Cambridgepark Dr, Cambridge, MA 02140

CTA: A 7T MRI Study (PI Bakshi) 12/31/19 – 12/12/24 0.12
Calendar EMD Serono, Inc. (Cumulative Total) 1.0% Effort
(Total Direct Costs)

The effect of cladribine on cerebral gray matter lesions and leptomeningeal involvement in multiple sclerosis: A 7T MRI study

This study's goal is to investigate how LME relates to GM lesions in relapsing-remitting (RR) MS at 7T.

Primary Objective: To assess the effect of newly started cladribine on the number of cortical lesions over two years.

Secondary Objectives: To assess the two-year effect of newly-started cladribine on the number of LME foci, thalamic lesions (number and volume), total cortical lesion volume, conventional brain MRI measures (T2 lesion

load, whole brain atrophy, parenchymal gadolinium-enhancing lesions), and conventional clinical measures (Expanded Disability Status Scale (EDSS) disability score, ambulation time, relapse rate).
Role: PI
Sponsor POC: Derek Main, derek.main@emdserono.com, 1-336-346-9199, 1 Technology Pl, Rockland MA 02370

RR 2005-A-13 (G-1510-06785) (PI Weiner) 10/01/16 – 09/30/21 0.60 Calendar
NMSS (Cumulative Total) 5.0% Effort
(Annual Direct Costs)
SUMMIT: An investigation of deeply phenotyped cohorts to understand disease outcomes and the biology of progression in MS
Our goal is to extend and develop an existing shared cohort of deeply phenotyped patients. Secondly, we will place this SUMMIT cohort at the center of a new platform and resource to be made accessible to other investigators interested in MS disease disability and progression.
Year 1: Aim 1) Expand and maintain the SUMMIT cohort, Aim 2) Create a platform resource for use by other investigators, Aim 3) MRI transfers and processing, Aim 4) Manuscript preparation(s)
Year 2: Aim 1) Integration of the genetics core, Aim 2) Joint MRI processing.
Role: Co-Investigator
Sponsor POC: Bruce Bebo PhD, bruce.bebo@nmss.org, (212) 476-0477, 733 Third Ave, New York, NY

W81XWH-18-1-0648 (PI Chitnis) 09/01/18 – 08/30/21 0.60
Calendar DOD/CDMRP (Cumulative Total) 5.0% Effort
(Annual Direct Costs)
Study of immune-based biomarkers using the longitudinal CLIMB dataset
Our hypothesis is that inflammatory or progressive activity in MS patients can be correlated to specific biomarkers which reflect relative involvement of adaptive, innate or neurodegenerative mechanisms.
Aim 1: Development of blood biomarkers for MS disease staging using both clinical and MRI measures.
Aim 2: Development of biomarker predicts of short and long-term outcomes in MS.
Aim 3: Development of composite biomarker panels and predictive models of disease outcomes using logistic regression models and machine learning.
Role: Co-Investigator
Sponsor POC: Jason D. Kuhns, jason.d.kuhns.civ@mail.mil, (301) 682-5507, USAMRAA, 820 Chandler St, Fort Detrick MD

RG-1707-28586 (PI Shinohara) 04/01/18 – 03/31/21 0.72
Calendar NMSS (Cumulative Total – Sub) 6.0% Effort
(Annual Direct Costs – Sub)
A traveling subject study of replicability in conventional and advanced MRI MS biomarkers
Dr. Bakshi and the BWH site will be responsible for patient recruitment, scan acquisition, and scan analysis. He and the postdoctoral fellow will serve as a central image analysis core, focusing on manual lesion identification and automated global and regional volumetrics. BWH will be responsible for recruiting and enrolling study subjects for participation, obtaining informed consent, and scheduling and performing all study visits according to the protocol.
Role: Co-Investigator, Consortium PI (BWH)
Sponsor POC: Walter Kostich, Ph.D., walter.kostich@nmss.org, (212)-476-0428, 733 Third Ave, New York, NY

W81XWH1910836 (PI Singhal) 09/30/19 - 09/29/21 0.30
Calendar Department of Defense/CDMRP (Cumulative Total) 2.5% Effort
(Annual Direct Costs)
Role of microglial activation and norepinephrine transporter abnormalities in pathogenesis of MS-related fatigue

The primary objective of this proposal is to assess the role of microglial activation and norepinephrine transporter (NET) binding in pathogenesis of MS-related fatigue, using novel Positron Emission Tomography (PET) radiotracers. [F-18]PBR06 and [C-11]MRB. The secondary objective is to determine the relationship of microglial activation and NET binding, with grey matter pathology (lesion load and brain atrophy) assessed using 7T MRI and evaluate their independent contribution in development of MS-related fatigue. We'll also perform an exploratory analyses on the relationship of [F-18]PBR06 PET and [C-11]MRB-PET with serum measurements of IL-1β, TNF-α, IL-6, MCP-1, MIF-1 and serum norepinephrine.
Aim 1: To determine the relationship of cerebral microglial activation, as assessed by [F-18]PBR06 PET, with MS-related fatigue.
Aim 2: To determine the relationship of norepinephrine transporter (NET) binding, as assessed by [C-11]MRB PET, with MS-related fatigue.
Aim 3: To determine the relationship of microglial activation and NET binding, with grey matter pathology (lesion load and brain atrophy) assessed using 7T MRI and evaluate their independent contribution in development of MS-related fatigue.
Role: Co-Investigator
Sponsor POC: Mark Wilkison, mark.d.wilkison.civ@mail.mil, 301-619-9883, U.S. Army Medical Research Acquisition Activity, 820 Chandler Street, Fort Detrick, MD, 21702

OVERLAP

There is no scientific or budgetary overlap.

RESEARCH SUPPORT

CHITNIS, Tanuja, MD, FAAN

ACTIVE

CTA: HLW-MS-PO-002 (PI Chitnis) 10/24/19 – 10/23/24
Tiziana Life Sciences (Cumulative) 0.88 Calendar
(Total Direct) 7.4% Effort *A Phase I Study of the Safety, Tolerability, and Immune Effects of the Oral Anti-CD3 Monoclonal Antibody Foralumab in Healthy Human Volunteers*
The primary objective of this study is to establish the safety of a single dose administration of oral foralumab to healthy volunteers, in escalating doses.
Role: PI
Sponsor POC: E. Priya Eddy, PhD, DABT, peddy@tizianalifesciences.com, 267-893-6747, 3805 Old Easton Rd., Doylestown, PA 18902

CTA: BCT-101-US (PI Chitnis) 10/17/18 – 10/16/24
BrainStorm Cell Therapeutics Ltd. (Cumulative) 0.12 Calendar
(Annual Direct) 1.0% Effort *A Phase 2, Open-Label, Multicenter Study to Evaluate Safety and Efficacy of Repeated Administration of NurOwn® (Autologous Mesenchymal Stem Cells Secreting Neurotrophic Factors; MSC-NTF Cells) in Participants with Progressive with Multiple Sclerosis*
The primary objectives of this study are to evaluate safety and tolerability of three intrathecal doses of NurOwn® (autologous MSC-NTF cells) given two months apart to participants with progressive MS.
Role: PI
Sponsor POC: Susan Ward, PhD, sward@brainstorm-cell.com, 339-234-3881, 1325 Avenue of Americas, 28th Floor, New York City, NY 10019

CTA: AG-031339JL (PI Healy) 06/19/19 – 06/18/24
Analysis Group, Inc. (Cumulative) 0.58 Calendar
(Annual Direct) 4.9% Effort
Prognostic Factors for Disease Progression in Multiple Sclerosis
The purpose of this proposed collaborative study is to assess the prognostic value of clinical factors beyond EDSS/CPD in multiple sclerosis, yielding important knowledge for clinical research and practice, and potentially informing improved economic analyses in MS.
Aim 1: Assess prognostic factors for disease progression in multiple sclerosis, and the extent to which factors beyond CDP contribute prognostic value for clinically meaningful disease milestones
Aim 2: Assess the relationship between quality of life metrics and EDSS, and the extent to which factors beyond EDSS contribute to quality of life
Aim 3: Evaluate implications for disease monitoring and economic modeling in multiple sclerosis
Role: Co-Investigator
Sponsor POC: Elyse Swallow, elyse.swallow@analysisgroup.com, 617-425-8483, 111 Huntington Ave., 14th Floor, Boston, MA 02199

R01AG067019 (PI Goldstein) 09/15/19 – 05/31/24
NIH/NIA 0.29 Calendar
2.4% Effort
Impact of Depression on Alzheimer's disease: Prenatal Immune Origins and Shared Impact of Sex

AD is associated with major depressive disorder (MDD), and both have twice the frequency in women than men. However, the shared pathophysiology that underlies MDD and AD is not well known. We will test the hypothesis that this shared risk has fetal origins that involve abnormalities in immune-stress and vascular pathways with sex-dependent consequences. We are uniquely poised to examine this *for the first time in humans* using our 60-year prospective prenatal cohort of adults, followed since 2nd/3rd trimesters of gestation and now ages 54-61. We will test the hypothesis that this shared risk has fetal origins involving abnormalities in immune-stress and vascular pathways with sex-dependent consequences.
Aim 1: Test whether prenatal immune biomarkers predict sex differences in early AD-related pathology.
Aim 2: Test whether prenatal immune markers (in Aim 1) associate with midlife neurovascular dysfunction.
Role: Co-Investigator, Site PI
Sponsor POC: Luci Roberts, roberlu@mail.nih.gov, 301-496-9350, Building 31, Room 5C27, 31 Center Drive, MSC 2292, Bethesda, MD 20892

CTA: HEORUSV201178 (PI Chitnis) 03/15/19 – 03/14/24
Novartis Pharma AG 0.12 Calendar
1.0% Effort *Relationship of Neurofilament Biomarker with Health Outcomes for MS Patients in CLIMB Registry*
The aim of this analysis is to examine the association between serum neurofilament (sNfL) levels and health outcomes (quality of life, persistence to index DMTs and healthcare resource utilization) in multiple sclerosis patients. The primary objective is to examine the association between sNfL levels and QoL measured by the Multiple Sclerosis Quality of Life 54 (MSQOL54) scale at baseline (at first sNfL visit in the assessment period). The secondary objective is to examine the association between baseline sNfL levels and QoL measures (given below) at baseline.
Role: PI
Sponsor POC: Chinmay Deshpande, PhD, Chinmay.Deshpande@novartis.com, 862-778-7512, One Health Plaza, 135/233C, East Hanover, NJ 07936

CTA: HEORUSV201134 (PI Chitnis) 11/30/18 – 11/29/23
Novartis Pharma AG 0.12 Calendar
1.0% Effort *Characterization of work productivity in relapsing MS patients over time using real-world data*
Previous research has demonstrated that RMS patients have considerable burden of lost work productivity, as captured by the Work Productivity and Impairment questionnaire (WPAI). The WPAI is widely used to measure the self-reported effect of health conditions and symptom severity on work productivity and regular activities during the past seven days.
Aim 1: Examine work productivity and activity impairment in relapsing multiple sclerosis patients over time (at Year 3) by using the WPAI:GH (general health) questionnaire
Aim 2: Identify associations between changes in the work productivity and clinical and patient-centric outcomes in the real-world.
The primary objective is to examine the overall work productivity change among the patients who improved, stayed the same, deteriorated on EDSS over time.
Role: PI
Sponsor POC: Chinmay Deshpande, PhD, Chinmay.Deshpande@novartis.com, 862-778-7512, One Health Plaza, 135/233C, East Hanover, NJ 07936

CTA: IIR5163 (PI Chitnis) 11/09/18 – 11/08/23
Mallinckrodt ARD, Inc. 0.06 Calendar
0.5% Effort *Effects of relapses on longterm outcomes in MS: CLIMB Study*
The overall goal of this study is to evaluate the impact of relapse severity and recovery on long-term outcomes in MS, and to identify biomarkers of relapse severity. In Aim 1 of this study, we will evaluate the effect of relapse severity and recovery on long-term outcomes in the CLIMB study including EDSS scores, timed 25-foot walk,

SDMT scores, and health-related quality of life measures. In Aim 2 of the study, we will explore associations of proteomic biomarkers of relapse severity and recovery on long-term outcomes in MS.
Aim 1: Identify patient characteristics and biomarkers associated with relapse severity and recovery in MS.
Aim 2: To evaluate the effects of early relapse severity and relapse recovery on 10-year outcomes in MS.
Role: PI
Sponsor POC: Catherine Brown, RN, catherinem.brown@mnk.com, 908-238-5525, 1425 Rt. 206, 2nd Floor, Bedminster, NJ 07921

CTA: CFTY720D2311 PARADIGMS (PI Chitnis) 02/24/14 – 05/31/23
Novartis Pharmaceuticals Corporation 0.12 Calendar
1.0% Effort *FTY720D2311 – A two-year, randomized, multicenter, active-controlled study to evaluate the safety and efficacy of fingolimod administered orally once daily versus interferon ß-1a i.m. once weekly in pediatric patients with multiple sclerosis.*
The purpose of this project is to evaluate the safety and efficacy of fingolimod versus interferon ß-1a i.m. in pediatric patients with MS. Assess the frequency of relapses by the ARR, number of new/newly enlarged T2 lesions, and the frequency and nature of adverse events as a measure of safety and tolerability.
Role: PI
Sponsor POC: Christine Kogel, RN, christine.kogel@novartis.com, 802-333-3676, One Health Plaza, East Hanover NJ 07936

R01AG057505 (PI Goldstein) 06/15/18 – 02/28/23
NIH/NIA 0.60 Calendar
5.0% Effort *Aging of Emotion Circuitry: Impact of Sex, Depression, and Fetal Immune Origins*
Maintaining emotional stability in the face of negative life experiences is critical for healthy aging. Maladaptive responses to negative affective stimuli ("NAffecS") are implicated in psychiatric disorders, including major depressive disorder (MDD). In fact, there are shared brain regions involved in responses to NAffecS and MDD pathophysiology, including: hypothalamus (HYPO), amygdala (AMYG), hippocampus (HIPP), anterior cingulate cortex (ACC), and ventromedial, ventrolateral, dorsolateral, and orbital prefrontal cortices (PFC), areas that are among the most sexually dimorphic. Activity in this circuitry is physiologically associated with cortisol response, loss of parasympathetic cardiac tone, and immune responses, including TNF-α, IL-1β, IL-6, responses that differ by sex. We will test here that immune pathway abnormalities, beginning in fetal development, are associated with sex-dependent impacts on HYPO, HIPP, AMYG and PFC, resulting in maladaptive negative emotion processing and MDD in early midlife. Further, aging of NAffec circuitry (i.e., decline) into later midlife will be accelerated by MDD in early midlife, resulting in greater deficits in negative emotion processing in later midlife, that we predict will differ by sex, i.e. women worse than men.
Role: Co-Investigator
Sponsor POC: Lisbeth Nielsen, nielsenli@nia.nih.gov, 301-402-4156, Building 31, Room 5C27, 31 Center Drive, MSC 2292, Bethesda, MD 20892

WBI Project 2017 (PI Chitnis) 10/15/17 – 10/14/21 BWH
Program for Interdisciplinary Neuroscience *Role of endocrine disruptors in the initiation of multiple sclerosis* 2.45 Calendar
20.4% Effort
The goal of this study is to:

1. Investigate whether endocrine disruptors including BPA and PBB are increased in females with MS.
2. To investigate the effects of endocrine disruptors on immune cells and their ability to migrate into the brain to cause neuro-autoimmune disorders including MS.
3. The interaction of endocrine disruptors with sex hormones in activating immune cells to cause neuro-autoimmune disorders including MS.

Role: PI

Sponsor POC: LaShaunda Gayden, lgayden@bwh.harvard.edu, 617-525-5595, 60 Fenwood Rd., 9002P, Boston, MA 02115

RR 2005-A-13 (G-1510-06785) (PI Weiner) 10/01/16 – 09/30/21
National MS Society 0.62 Calendar
5.2% Effort

SUMMIT: An investigation of deeply phenotyped cohorts to understand disease outcomes and the biology of progression in MS

Our goal is to extend and develop an existing shared cohort of deeply phenotyped patients. Secondly, we will place this SUMMIT cohort at the center of a new platform and resource to be made accessible to other investigators interested in MS disease disability and progression.

Year 1: Aim 1) Expand and maintain the SUMMIT cohort, Aim 2) Create a platform resource for use by other investigators, Aim 3) MRI transfers and processing, Aim 4) Manuscript preparation(s)

Year 2: Aim 1) Integration of the genetics core, Aim 2) Joint MRI processing.

Role: Co-Investigator

Sponsor POC: Bruce Bebo, PhD, bruce.bebo@nmss.org, 212-476-0477, 733 3rd Ave., New York, NY 10017

W81XWH-18-1-0648 (PI Chitnis) 09/01/18 – 08/31/21
DOD/CDMRP 1.80 Calendar
15.0% Effort *Study of immune-based biomarkers using the longitudinal CLIMB dataset*

Our hypothesis is that inflammatory or progressive activity in MS patients can be correlated to specific biomarkers which reflect relative involvement of adaptive, innate or neurodegenerative mechanisms.

Aim 1: Development of blood biomarkers for MS disease staging using both clinical and MRI measures.

Aim 2: Development of biomarker predicts of short and long-term outcomes in MS.

Aim 3: Development of composite biomarker panels and predictive models of disease outcomes using logistic regression models and machine learning.

Role: PI

Sponsor POC: Ebony Simmons, Ebony.s.simmons.civ@mail.mil, 301-619-2105, USAMRAA, 820 Chandler St., Ft. Detrick, MD 21702

R01NS098023 (PI Xia) 09/01/16 – 06/30/21
NIH/NINDS 0.12 Calendar
1.0% Effort *Integrating EHR and Genomic to Predict Multiple Sclerosis Drug Response*

The overall goal of this project is to leverage electronic health records (EHR) data to define treatment response and integrate clinical features from EHR data with genomics data to improve prediction of treatment response in MS. Dr. Tanuja Chitnis is the site PI and will advise Dr. Xia (PI, University of Pittsburgh) on clinical guidance as pertaining to this proposal. Dr. Chitnis has expertise in clinical and translational research in multiple sclerosis. Dr. Chitnis will supervise the research fellow embedded in the Partners MS Center who reviews medical records to establish gold standard patient subset, and interface with the Partners Research Computing team that generates the electronic health records data mart.

Aim 1: Leverage narrative electronic health records data (e.g., clinical notes, radiology reports) and natural language processing (NLP) to ascertain individualized response to DMTs (n=600 for each DMT).

Aim 2: Identify clinical features from electronic health record data (e.g., diagnoses, exposures) that predict response to DMTs using a systematic phenome-wide approach.

Aim 3: Develop and test a comprehensive predictive model of individualized response to DMTs that incorporates clinical and genetic predictors.

Role: Co-Invstigator, Site PI

Sponsor POC: Ursula Utz, PhD, utzu@ninds.nih.gov, 301-496-143, NINDS - Neuroscience Center, Division of Extramural Activities, 6001 Executive Boulevard Suite 3309, Bethesda, MD 20892- 9531

CLIMB A2 (PI Chitnis) 05/27/16 – 05/26/21
EMD Serono, Inc. 0.06 Calendar
0.5% Effort

CLIMB Study Addendum No. 2
A retrospective analysis comparing Tecfidera to Rebif has been requested. Plan overview: Conduct query on NEDA-2 (relapse and new MRI lesions (T2+Gd)) on Rebif vs. Tecfidera subjects.
Role: PI
Sponsor POC: Erin Teetshorn, erin.teetshorn@emdserono.com, 781-206-0050, 1 Technology Pl, Rockland MA 02370

NPMSC-2019 (PI Chitnis) 07/01/19 – 06/30/22
National Pediatric MS Consortium 0.90 Calendar
7.5% Effort

NPMSC Steering Committee Chair (2019 – 2020)
Dr. Chitnis will serve as the Chair of the National Pediatric MS Consortium (NPMSC) commencing July 1, 2019 for one year. This project will support her effort in responsibilities related to acting as the Chair.
Role: PI
Sponsor POC: (prime institution) Shelly Roalstad, shelly.roalstad@hsc.utah.edu, 801-587-7565, 295 Chipeta Way, Salt Lake City, UT 84108

2019A011503 (PI Goldstein) 06/01/19 – 05/31/21
John Sperling Foundation (Sub Cumulative) 0.36 Calendar
(Sub Total Direct) 3.0% Effort *Sex*

Differences in Alzheimer's disease: The Critical Role of Brain Metabolism
This is a supplemental project to the BrightFocus Foundation grant to enhance the understanding of sex differences in the risk for AD regarding brain metabolic function. Mitochondria is responsible for converting glucose to energy in cells, highly regulated by estradiol, and less efficient with age, in particular for women after menopause. Our team has acquired state-of-the-art new technology to allow us to directly assess mitochondrial function in immune cells. Our team will process blood samples from subjects, test for mitochondrial function, and collect and analyze data. The grant will allow us to investigate this critical pathway for the clinical risk algorithm for AD, which will contribute to understanding sex differences in memory decline and risk of AD.
Role: Co-Investigator, Site PI
Sponsor POC: (prime institution) Jill Goldstein, jill_goldstein@hms.harvard.edu, 617-724-1904, MGH, 55 Fruit St., Boston, MA 02114

HC-1411-02009 (PI Chitnis) 10/01/15 – 03/31/21 NCE
National MS Society (Cumulative) 0.12 Calendar
(Annual Direct) 1.0% Effort *Patient-*

family views on pediatric MS research needs, outcomes, and methods
The goal of this project is to gather the opinion on pediatric MS research priorities from parents of children with MS, teenagers with MS, and adults with pediatric-onset MS.
Role: PI
Sponsor POC: Nicholas LaRocca, PhD, nicholas.larocca@nmss.org, 212-443-4299, 733 3rd Ave, New York, NY 10017

CA2018607 (PI Goldstein) 03/30/18 – 03/28/21
BrightFocus Foundation NCE (Cumulative) 0.60 Calendar
(Annual Direct) 5.0% Effort

Development of Clinical Algorithm to Identify Risk of Alzheimer's Disease in Early Midlife
Aim 1: Assess critical components of an enhanced clinical risk algorithm for AD/amyloid accumulation and memory circuitry deficits in early midlife and begin validation process - a tool that could be deployed to identify who to treat early to potentially attenuate AD disability later in life or, ultimately, prevent the illness.

Aim 2: Assess effect sizes for types of phenotyping most strongly associated with structural and functional changes in memory circuitry regions and amyloid accumulation in asymptomatic individuals in early midlife.
Aim 3: Evaluate new phenotyping technologies for their putative role in enhancing the risk algorithm.
Role: Co-Investigator
Sponsor POC: (prime institution) Jill Goldstein, jill_goldstein@hms.harvard.edu, 617-724-1904, MGH, 55 Fruit St., Boston, MA 02114

CTA: EFC11759 (TERIKIDS) (PI Chitnis) 02/18/15 – 12/31/20
Sanofi US Services, Inc. (Cumulative) 0.12 Calendar
(Annual Direct) 1.0% Effort *A two year, multicenter, randomized, double-blind, placebo-controlled, parallel group trial to evaluate efficacy, safety, tolerability, and pharmacokinetics of teriflunomide administered orally once daily in pediatric patients with relapsing forms of multiple sclerosis (TERIKIDS)*
The primary objective is to assess the effect of teriflunomide in comparison to placebo on disease activity measured by time to first clinical relapse after randomization in children and adolescents 10 to 17 years of age with relapsing forms of MS.
Role: PI
Sponsor POC: April Cuccia, april-ext.cuccia@sanofi.com, 908-981-5653, 55 Corporate Drive, Bridgewater, NJ 08807

OVERLAP

There is no scientific overlap or budgetary overlap. Currently, Dr. Chitnis' effort stands at 10.04 CM. Further effort assessment will occur should pending project(s) be funded, in conjunction with sponsor/agency staff.

RESEARCH SUPPORT

HEALY, Brian C., PhD

ACTIVE

UL1TR002541 (PI Nadler) 05/01/18 – 04/30/23 4.60 Calendar
NIH/NCATS (NCRR) 38.33% Effort

Harvard Clinical and Translational Science Center
Harvard Catalyst Workforce Development and Academy programs (Non-Federal)
Provide enriched resources to educate and develop the next generation of researchers trained in the complexities of translating research discoveries into clinical trials and ultimately, into practice. Design new and improved clinical research informatics tools for analyzing research data and managing clinical trials. Support outreach to underserved populations, local community and advocacy organizations, and health care providers. Assemble interdisciplinary teams and forge new partnerships with private and public health care organizations.
Role: Co-Investigator
Sponsor POC: Mary E. Purucker, M.D., Ph.D., puruckerm@mail.nih.gov, (301) 435-0741, NCATS, 6701 Democracy Boulevard, Bethesda MD 20892-4874

P50MH115846 (MPI Öngür/Hsu/Hernan) 05/15/19 – 03/31/23 2.70 Calendar
NIH/NIMH 22.5% Effort

Laboratory for Early Psychosis Research (LEAP)
The goal of this proposal is to establish a Center that collects standardized data and uses state-of-the-art analysis techniques to enhance our understanding of heterogeneity in patient outcomes and treatment approaches in first episode psychosis.
Aim 1 Data Synthesis: To acquire and integrate existing data sets, including the Massachusetts All Payer Database (APCD) and the clinical data on all FEP patients seen in clinics participating in the Massachusetts DMH Center of Excellence and the LEAP Center. The Methods Core will coordinate the integration of standardized clinical measures and their linkage to existing healthcare databases, and will create a novel, integrated data repository to help address clinical questions in FEP and mental health research in general.
Aim 2 Clinical Prediction: To develop the infrastructure to support the use of all existing data for clinical prediction. The Methods Core will provide support to implement recent technological advances such as machine learning algorithms for all Center activities that include a clinical prediction component.
Aim 3. Comparative Effectiveness Research: To develop the infrastructure to support the use of all existing data to compare the effectiveness of different clinical management strategies. The Methods Core will provide support to implement causal inference methods and related techniques for all Center activities that include a causal inference component.
Role: Co-Investigator
Sponsor POC: Matthew V. Rudorfer, M.D., mrudorfe@mail.nih.gov, 301-443-1111, The National Institute of Mental Health – 6001 Executive Boulevard, Room 7137, MSC 9635, Bethesda, MD 20892

R01HL133149 (PI Huffman) 07/01/17 – 04/30/22 0.30
Calendar NIH/NHBLI 2.5% Effort

Pragmatic Collaborative Care for Cardiac Inpatients with Depression or Anxiety
The goal of this project is to assess the impact of a multipronged collaborative care management program for patients with depression or anxiety disorders among patients admitted for heart failure or an acute coronary syndrome.

Aim 1: Measure will be physical function at 26 weeks, given that low function independently predicts new cardiac events. Our novel approach targeting multiple contributors to low function in ACS/HF patients should have strong effects on this main outcome.
Aim 2: Examine group differences on additional key patient-reported outcomes and cost metrics, and we will assess the intervention's impact on major cardiac events throughout the study period.
Role: Co-Investigator
Sponsor POC: Catherine Stoney, Ph.D., catherine.stoney@nih.gov, 301-435-6670, NHBLI, Building 31, 31 Center Drive, Bethesda, MD 20892

R21NR018738 (PI Celano) 04/01/20 – 03/31/22 0.30
Calendar NIH/NINR 2.5% Effort

An adaptive personalized text message intervention for cardiac prevention
Aim 1: To assess the feasibility and acceptability of the adaptive TMI.
Aim 2: To compare between-group improvements in psychological health (e.g., positive affect) and health behaviors (via composite index) at 12 weeks (primary time point) and 24 weeks.
Aim 3: To explore improvements in health-related outcomes (e.g., physical function, blood pressure, lipids, and AHA cardiac risk score) between groups at 12 and 24 weeks.
Role: Biostatistician
Sponsor POC: Sung Sug Yoon, sungsug.yoon@nih.gov, (301) 402-6959, 6701 Democracy Blvd., Room 710, One Democracy Plaza, Bethesda, MD 20892-4870

RR 2005-A-13 (G-1510-06785) (PI Weiner) 10/01/16 – 09/30/21 0.48 Calendar
NMSS 4.0% Effort

SUMMIT: An investigation of deeply phenotyped cohorts to understand disease outcomes and the biology of progression in MS
Our goal is to extend and develop an existing shared cohort of deeply phenotyped patients. Secondly, we will place this SUMMIT cohort at the center of a new platform and resource to be made accessible to other investigators interested in MS disease disability and progression.
Year 1 – Aim 1: Expand and maintain the SUMMIT cohort, Aim 2: Create a platform resource for use by other investigators, Aim 3: MRI transfers and processing, Aim 4: Manuscript preparation(s)
Year 2 – Aim 1: Integration of the genetics core, Aim 2: Joint MRI processing.
Role: Co-Investigator
Sponsor POC: Bruce Bebo, PhD, bruce.bebo@nmss.org, 212-476-0477, 733 3rd Ave., New York, NY 10017

PP-2001-35512 (PI Cavallari) 10/01/20 – 09/30/21 0.18 Calendar
NMSS 1.5% Effort

Pilot study evaluating the effect of small vessel disease on disease progression in multiple sclerosis
The overall objective of this study is to estimate the effect of small vessel disease (SVD) on disease progression in multiple sclerosis (MS).
Aim 1: To estimate the association between retinal biomarkers of SVD, including additional measures of arteriolar and venular diameter and tortuosity, and clinical measures of MS disease worsening and inflammatory activity in longitudinal analysis.
Aim 2: To estimate the association between retinal biomarkers of SVD and imaging measures of MS disease progression and inflammatory activity using longitudinal magnetic resonance imaging (MRI) and OCT data.
Aim 3: To estimate the difference in association of SVD with clinical and imaging measures across subgroups of patients stratified by age, sex, disease duration, disease type, and vascular risk factors to refine our inclusion and exclusion criteria and assess recruitment potential for future single- or multi-center studies.
Role: Co-Investigator
Sponsor POC: Fiona Brabazon, Fiona.Brabazon@nmss.org, 212-476-0475, 733 3rd Ave., New York, NY, 10017

W81XWH-18-1-0648 (PI Chitnis) 09/01/18 – 08/31/21 0.60
Calendar DOD/CDMRP

Study of immune-based biomarkers using the longitudinal CLIMB dataset
Our hypothesis is that inflammatory or progressive activity in MS patients can be correlated to specific biomarkers which reflect relative involvement of adaptive, innate or neurodegenerative mechanisms. Aim 1: Development of blood biomarkers for MS disease staging using both clinical and MRI measures. Aim 2: Development of biomarker predicts of short and long-term outcomes in MS. Aim 3: Development of composite biomarker panels and predictive models of disease outcomes using logistic regression models and machine learning.
Role: Co-Investigator
Sponsor POC: Ebony Simmons, Ebony.s.simmons.civ@mail.mil, 301-619-2105, USAMRAA, 820 Chandler St., Ft. Detrick, MD 21702

1-17-ICTS-099 (PI Huffman) 01/01/17 – 12/31/20 0.00 Calendar
American Diabetes Association 0.0% Effort

A novel psychological-behavioral intervention to increase activity in type 2 diabetes
Goal: To develop and test a combined positive psychology-motivational interviewing intervention to improve activity and other health behaviors in patients with type 2 diabetes via proof-of-concept and feasibility trials.
Role: Biostatistician
Sponsor POC: Orville Kolterman, 2451 Crystal Drive, Suite 900, Arlington, VA 22202

ML39789 (PI Weiner) 05/15/17 – 12/31/20 0.00
Calendar Genentech, Inc. 0.0% Effort

CTA: Immune Profiling During Ocrelizumab Treatment in Multiple Sclerosis
The goal of this study is to perform immune profiling in patients with MS initiating treatment with ocrelizumab before and during treatment with the goal to better understand how B cell depletion affects other cell types within the immune system with a focus on the myeloid compartment, antibody signatures, and miRNA profiles.
Role: Biostatistician
Sponsor POC: Mark Cabatingan, cabatingan.mark-s@gene.com, 401-475-5372, 600 Massachusetts Ave NW #300, Washington, DC 20001

AG 031339JL (PI Healy) 06/19/19 – 11/30/20 2.72
Calendar Analysis Group, Inc. 22.67% Effort

CTA: Prognostic Factors for Disease Progression in Multiple Sclerosis
The purpose of this proposed collaborative study is to assess the prognostic value of clinical factors beyond EDSS/CPD in multiple sclerosis, yielding important knowledge for clinical research and practice, and potentially informing improved economic analyses in MS.
Aim 1: Assess prognostic factors for disease progression in multiple sclerosis, and the extent to which factors beyond CDP contribute prognostic value for relapse, disability progression, or conversion to secondary progressive multiple sclerosis (SPMS)
Aim 2: Assess the relationship between quality of life metrics and MRI metrics, and quality of life metrics and EDSS, and the extent to which factors beyond EDSS contribute to quality of life
Aim 3: Evaluate implications for disease monitoring and economic modeling in multiple sclerosis
Role: PI
Sponsor POC: Elyse Swallow, elyse.swallow@analysisgroup.com, 617-425-8483, 111 Huntington Ave., 14th Floor, Boston, MA 02199

OVERLAP

There is no scientific or budgetary overlap.

RESEARCH SUPPORT

PATSOPOULOS, Nikolaos, M.D., Ph.D.

ACTIVE

JF-1808-32223 (PI Patsopoulos) 07/01/19 – 06/30/246.00 Calendar

National Multiple Sclerosis Society, Harry Weaver Award 50.0% Effort

Omic-based precision medicine strategies in multiple sclerosis
In this application, we aim to develop and refine methods to translate the large number of MS-associated genetic variants for diagnosis and prognosis in various clinical settings. To achieve this overarching objective, the proposal encompasses three ongoing research pillars: i) continuous efforts to refine the genetic architecture of MS; ii) functional characterization of MS variants; and iii) quantification of diagnostic and predictive utility of MS-associated genetic variants.
Aim 1: We will further refine genetic associations in MS.
Aim 2: We will re-purpose data from ongoing efforts to functionally characterize the effect of MS-associated genetic variants in various cell types
Aim 3: We will quantify the diagnostic and prognostic utility of the above GRSs in different clinical settings, leveraging population-level large-scale biobanks with genetic data and longitudinal cohorts of MS individuals
Role: PI
Sponsor POC: Nicholas LaRocca, PhD, nicholas.larocca@nmss.org, (212) 443-4299, 733 Third Ave, New York, NY

RG-1707-28657 (PI Patsopoulos) 04/01/18 – 03/31/21 2.40 Calendar
National Multiple Sclerosis Society 20.0% Effort

Sex specific genetics of multiple sclerosis
We propose to advance our understanding of the role of sex in genetics of MS by applying a systematic and agnostic approach. Aim 1: Identify sex-specific MS genetic variants; Aim 2: Functional annotation and identification of implicated pathways; Aim 3: In-depth investigation of sex chromosome associations in MS
Role: PI
Sponsor POC: Nicholas LaRocca, PhD, nicholas.larocca@nmss.org, (212) 443-4299, 733 Third Ave, New York, NY

W81XWH1810648 (PI Chitnis) 09/01/18 – 08/31/21 0.60
Calendar DOD/CDMRP 5.00% Effort

Study of immune-based biomarkers using the longitudinal CLIMB dataset
Our hypothesis is that inflammatory or progressive activity in MS patients can be correlated to specific biomarkers which reflect relative involvement of adaptive, innate or neurodegenerative mechanisms.
Aim 1: Development of blood biomarkers for MS disease staging using both clinical and MRI measures.
Aim 2: Development of biomarker predicts of short and long-term outcomes in MS.
Aim 3: Development of composite biomarker panels and predictive models of disease outcomes using logistic regression models and machine learning.
Role: Co-Investigator
Sponsor POC: Jason D. Kuhns, jason.d.kuhns.civ@mail.mil, (301) 682-5507, USAMRAA, 820 Chandler St, Fort Detrick MD

RR 2005-A-13 (G-1510-06785) (PI Weiner) 10/01/16 – 09/30/21 0.60 Calendar
National Multiple Sclerosis Society 5.00% Effort

SUMMIT: An investigation of deeply phenotyped cohorts to understand disease outcomes and the biology of progression in MS
Our goal is to extend and develop an existing shared cohort of deeply phenotyped patients. Secondly, we will place this SUMMIT cohort at the center of a new platform and resource to be made accessible to other investigators interested in MS disease disability and progression.
Year 1: Aim 1) Expand and maintain the SUMMIT cohort, Aim 2) Create a platform resource for use by other investigators, Aim 3) MRI transfers and processing, Aim 4) Manuscript preparation(s)
Year 2: Aim 1) Integration of the genetics core, Aim 2) Joint MRI processing.
Role: Co-Investigator
Sponsor POC: Bruce Bebo, PhD, bruce.bebo@nmss.org, 212-476-0477, 733 3rd Ave., New York, NY 10017

PP-1905-34011 (PI Patsopoulos) 10/01/19 – 09/30/20 0.53 Calendar
National Multiple Sclerosis Society 4.41% Effort

In-depth multi-omic characterization of lesion and lesion-free brain tissue
We aim to establish the basis of an in-depth multi-omic map of the MS brain. We propose a systematic in-depth characterization of MS lesions, lesion boundaries, and lesion-free brain tissue leveraging cutting-edge technologies to generate tissue, cell-type, and single-cell level data
Aim 1: We will expand the type of data that we can extract from MS brain samples
Aim 2: We will optimize our MRI-guided sampling in order to study multiple affected and non-affected regions, allowing detailed classification of the MS lesions
Aim 3: We aim to leverage this pipeline to study a large sample of MS subjects and compare their brains vs. other neurodegenerative diseases and healthy controls
Role: PI
Sponsor POC: Douglas Landsman, Ph. D., douglas.landsman@nmss.org, 212-476-0536, 733 3rd Ave, New York, NY 10017

OVERLAP
There is no scientific or budgetary overlap.

RESEARCH SUPPORT

WEINER, Howard L., MD

ACTIVE

RF1 AG065270 (PI Weiner) 09/30/20 – 08/31/24 3.00 Calendar
NIH/NIA 25% Effort

The Role of Microbiota in Aging and Alzheimer's Disease
The goal of this proposal is to investigate the mechanisms by which intestinal microbiota influence aging and neurologic disease in the hopes of identifying new therapeutic targets for treatment of AD.
Aim 1: Which microbial factors alter AD pathogenesis in Aβ and tau animal models?
Aim 2: How does the microbiota modulate innate immunity Aβ and tau animal models?
Role: PI
Sponsor POC: Suzana Petanceska, petanceskas@nia.nih.gov, 301-496-9350, 10 Center Dr, Bethesda, MD 20814

RG-1907-34686 (PI Weiner) 04/01/20 – 03/31/23
National Multiple Sclerosis Society
The Role of B Cells in CNS Autoimmunity
Subproject – *Human B-Cells* 0.30 Calendar
2.5% Effort
Subproject – *Animals* 0.30 Calendar
2.5% Effort
In our proposal, we will study both inflammatory and regulatory B cell subsets in mice with EAE and patients with MS, and will specifically investigate 1) the relationship with other immune checkpoint inhibitors, 2) the effect of B-cell therapy (anti-CD20), and 3) the association with the intestinal microbiota.
Aim 1. Study the role that effector B cell (Beff)-derived cytokines play in the pathogenesis of MS and EAE.
Aim 2. Determine the molecular signature of defined Beff cell subpopulations in EAE and in MS, and whether the Beff subsets in MS are altered in function and transcriptome upon reconstitution after anti-CD20 depletion therapy.
Aim 3. Elucidate the molecular signature and functional activity of defined regulatory B cell subsets in the mouse and in MS patients that have and have not been treated with B cell-depleting, anti-CD20 therapy.
Role: PI
Sponsor POC: Fiona Brabazon, Fiona.Brabazon@nmss.org, 212-476-0475, 733 3rd Ave., New York, NY, 10017

R21 AG063187 (PI Weiner) 03/01/20 – 02/28/22 1.20 Calendar
NIH/NIA 10% Effort

Targeting CNS Neuroinflammation in Animal Models of Alzheimer's Disease
We will investigate the involvement of IL-10 in the anti-CD3 positive effects we observed in AD models, and the possibility that immunoregulation and immune activation play opposite roles at different stages of the disease.
Aim 1. Investigate the effect of nasal anti-CD3 on microglia phenotype and phagocytic function in aging.
Aim 2. Investigate the effect of nasal anti-CD3 in the 3xTg AD model
Role: PI
Sponsor POC: Lisa Opanashuk, lisa.opanashuk@nih.gov, 301-827-5422, 10 Center Dr, Bethesda, MD 20814

2020CureALZ: Weiner/Cox (PI Weiner) 02/15/20 – 02/14/22 0.60
Calendar Cure Alzheimer's Fund 5.0% Effort

Targeting the Microbiome and Innate Immunity in Alzheimer's Disease
We will investigate whether the microbiota from AD patients worsens amyloid-beta and tau pathology in animal models of AD, and test whether microbiota from young vs age-matched healthy controls has a greater protective effect.
Aim 1: Which microbial factors alter AD pathogenesis in Ab and tau animal models?
Aim 2: How does the microbiota modulate innate immunity in Ab and tau animal models?
Role: PI
Sponsor POC: Meg Smith, msmith@curalz.org, 781-237-3811, 34 Washington St., Suite 310, Wellesley Hills, MA 02481

RR 2005-A-13 (G-1510-06785) (PI Weiner) 10/01/16 – 09/30/21 0.60
Calendar National Multiple Sclerosis Society 1,311,250 (Cumulative Total)
5.0% Effort

SUMMIT: An Investigation of Deeply Phenotyped Cohorts to Understand Disease Outcomes and the Biology of Progression in MS
Our goal is to extend and develop an existing shared cohort of deeply phenotyped patients. Secondly, we will place this SUMMIT cohort at the center of a new platform and resource to be made accessible to other investigators interested in MS disease disability and progression.
Year 1: Aim 1) Expand and maintain the SUMMIT cohort, Aim 2) Create a platform resource for use by other investigators, Aim 3) MRI transfers and processing, Aim 4) Manuscript preparation(s)
Year 2: Aim 1) Integration of the genetics core, Aim 2) Joint MRI processing.
Role: PI
Sponsor POC: Bruce Bebo, PhD, bruce.bebo@nmss.org, 212-476-0477, 733 3rd Ave., New York, NY 10017

W81XWH-18-1-0648 (PI Chitnis) 09/01/18 – 08/31/21 0.60
Calendar DOD/CDMRP 5.0% Effort

Study of Immune-based Biomarkers Using the Longitudinal CLIMB Dataset
Our hypothesis is that inflammatory or progressive activity in MS patients can be correlated to specific biomarkers which reflect relative involvement of adaptive, innate or neurodegenerative mechanisms.
Aim 1: Development of blood biomarkers for MS disease staging using both clinical and MRI measures.
Aim 2: Development of biomarker predicts of short and long-term outcomes in MS.
Aim 3: Development of composite biomarker panels and predictive models of disease outcomes using logistic regression models and machine learning.
Role: Co-Investigator
Sponsor POC: Ebony Simmons, Ebony.s.simmons.civ@mail.mil, 301-619-2105, USAMRAA, 820 Chandler St., Ft. Detrick, MD 21702

RG-1707-28516 (MPI Weiner/Liu) 04/01/18 – 03/31/21 1.80
Calendar National Multiple Sclerosis Society (Cumulative Total) 15%
Effort

The Role of Fecal MicroRNAs in CNS Autoimmune Inflammatory Disease
Our hypothesis is the dynamic changes in fecal microRNAs during the course of EAE and in MS relate to immune mechanisms associated with modulating disease.
Aim 1: Investigation of fecal microRNAs in EAE
Aim 2: Effect of protective fecal microRNAs on the microbiome and immune mechanisms in EAE
Aim 3: Effect of synthetic microRNAs on immune function and the microbiome in EAE

Role: PI
Sponsor POC: Bruce Bebo, PhD, bruce.bebo@nmss.org, 212-476-0477, 733 3rd Ave., New York, NY 10017

R01 EY027921 (MPI Butovsky/Haider/Weiner) 09/01/17 – 12/31/20
NIH/NEI
Role of Microglia in Retinitis Pigmentosa
Project 2 1.20 Calendar
10% Effort The focus of this grant proposal is to characterize retinal microglia and how they regulate and/or participate in retinal damage in both animal models of retinitis pigmentosa and in eyes from human subjects. We will investigate whether retinitis pigmentosa in animal models can be treated by specifically targeting and modulating microglia. We will use new technology and approaches to understand features of microglia cells that can then be exploited to develop novel microglia-targeting therapies to treat humans with retinitis pigmentosa.
Aim 1. Identify molecular pathways affected in retinal microglia in mouse models and human RP.
Aim 2. Target the TREM2-APOE-SPP1 pathway to inhibit MGnD-cytotoxic microglia in rd mice.
Aim 3. Restore M0-homeostatic microglia via TGFβ1-MERTK signaling in rd mice.
Role: Co-PI
Sponsor POC: George Ann McKie, mckiegeo@mail.nih.gov, (301) 496-5248, National Eye Institute, 31 Center Drive MSC 2510, Bethesda, MD

BRI Next Gen Award (PI Cox) 11/25/19 – 11/24/20 0.12 Calendar
Brigham Research Institute 1.0% Effort

Investigating the Role of the Microbiome in Neurologic Diseases
The goal of this study is to identify bacteria and bacterial products in the microbiome that can ultimately be used to treat neurologic diseases and to identify related immune signaling mechanisms.
Aim 1: Which bacteria are associated with neurologic diseases?
Aim 2: How does the microbiota contribute to pathogenesis in animal models of neurologic disease?
Role: Co-Investigator
Sponsor POC: Anu Swaminathan, PhD, aswaminathan@partners.org, 617-525-8380, 75 Francis St., Boston, MA 02115

ACTIVE – NO EFFORT/SALARY

CTA: miRNA (PI Weiner) 08/10/16 – 08/09/21 0.00 Calendar
Genzyme Corp 0.0% Effort

CTA: miRNA Profiling in Teriflunomide (Aubagio) Treated Patients
The goal of this study is to identify signature of miRNA in serum/plasma and immune cells associated with therapeutic response to Teriflunomide.
Role: PI
Sponsor POC: Khac Huy Vo, Khachuy.vo@genzyme.com, 978-440-0327, 500 Kendall Street, Cambridge, MA 02142

CTA: MS (PI Weiner) 05/15/17 – 12/31/20 0.00 Calendar
Genentech, Inc. 0.0% Effort

Immune Profiling During Ocrelizumab Treatment in Multiple Sclerosis

The goal of this study is to perform immune profiling in patients with MS initiating treatment with ocrelizumab before and during treatment with the goal to better understand how B cell depletion affects other cell types within the immune system with a focus on the myeloid compartment, antibody signatures, and miRNA profiles.
Role: PI
Sponsor POC: Mark Cabatingan, cabatingan.mark-s@gene.com, 401-475-5372, 600 Massachusetts Ave NW #300, Washington, DC 20001

MassCATS (PI Hyman)	12/01/19 – 11/30/20	0.12 Calendar
Massachusetts Life Sciences Center	1.0% Effort	

Modulating Microglial Neuroinflammation with Anti-CD3 Monoclonal Antibody
The proposed study will determine if nasal administration of anti-CD3 monoclonal antibody may be a potential target for AD therapy by investigating its role in the 3xTg mouse model of AD. Because nasal anti-CD3 has been shown to induce IL-10 production by T cells and because IL-10 is an important anti-inflammatory cytokine that modulates microglial activation - which is critical for AD pathology - we will investigate microglial cells after nasal anti-CD3 treatment using high throughput RNA sequencing, histopathology and flow cytometric analysis.
Aim 1. determine the ability of nasal anti-CD3 to ameliorate AD-like pathology in 3xTg AD mice
Aim 2. perform complete behavioral characterization of nasal anti-CD3 on cognitive and motor functions both prior to and following treatment.
Aim 3. perform a gene expression analysis in resident microglia of brains from 3xTg AD mice.
Role: Consortium PI
Sponsor POC: Katherine Rauf, krauf@mgh.harvard.edu, 617-643-4279, 110 6th Street, Bldg 120-2, Charlestown, MA 02129

OVERLAP
There is no scientific or budgetary overlap.

- Serum neurofilament light (sNfL) is a candidate biomarker of neuroinflammation in patients with multiple sclerosis (MS)
- sNfL shows a high patient-to-patient variability, which has been attributed to recent disease activity, brain atrophy rates, and aging

OBJECTIVE

- To explore the association between sNfL and Age in patients with MS
- To explore the effect of Age on the association between Clinical Relapses/Gd+ Lesions and sNfL

METHODS

SUBJECTS

- We included 94 patients with MS enrolled in the Comprehensive Longitudinal Investigation of MS at the Brigham and Women's Hospital (**CLIMB**) study

SERUM NEUROFILAMENT LIGHT

- sNfL levels were measured at yearly intervals for ten years with a single-molecule array (SIMOA) assay

STATISTICAL ANALYSIS

- We used multivariable linear mixed-effects models with a random intercept to test the association between age and sNfL separately in three disease activity groups
- We included three different groups:
  - patients in sustained remission (>365 days)
  - patients with a recent clinical relapse (within 90 days)
  - patients with a recent brain Gd+ lesion (within 90 days)
- We also ran a separate model to assess the interaction of age and the Gd+/Gd- status and the interaction between age and relapse/remission status
- All analyses were adjusted for disease-modifying therapy use and sex

- (p=0.008) (**Figure 1**)
- The fourth age quartile showed 31.2% higher sNfL levels than the first age quartile (p=0.005)
- Patients with a recent clinical relapse had a 2.2% increase in sNfL per year (p=0.049) (**Figure 2**)
- sNfL was not significantly associated with age after a recent Gd+ lesion (p= 0.88)
- We assessed the interaction between Age and Gd+ lesion status where we observed a negative interaction (– 1.72% annual decrease, p=0.004) (**Figure 3**)

CONCLUSIONS

- Our findings show that the association between sNfL and Gd+ lesion status is modulated by Age
- We propose that younger patients experience a greater elevation in sNfL than older patients after a Gd+ lesion, which provides potential clues on neuroinflammation and aging in MS

Characteristics		MS patients (n=94)
Sex, n (%)	Female	69 (73%)
	Male	25 (27%)
Race, n (%)	White	90 (96%)
	African American	1 (1%)
	Unknown	2 (2%)
	More than one race	1 (1%)
Age, years at baseline (mean, SD)		37.4 ± 8.9
Disease duration, years at baseline (mean, SD)		2.3 ± 1.4
EDSS at baseline (median, interquartile range)		1.0 (0 – 2.0)
DMT use at first visit, n (%)	No DMT	24 (25.5%)
	Interferon beta-1a	39 (41.5%)
	Interferon beta-1b	5 (5.3%)
	Glatiramer acetate	23 (24.5%)
	Cyclophosphamide	1 (1.1%)
	Natalizumab	2 (2.1%)

Legend: DMT = disease-modifying therapy; EDSS= expanded disability status scale; MS= multiple sclerosis; n= patient count; SD= standard deviation

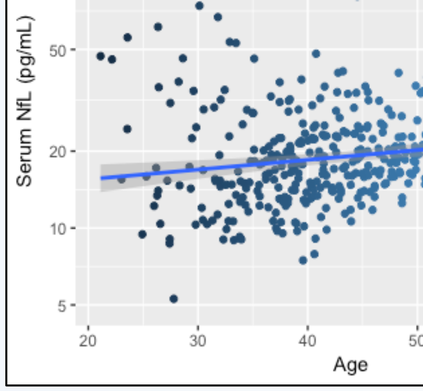


Figure 2: **sNfL dynamics in Remissio**

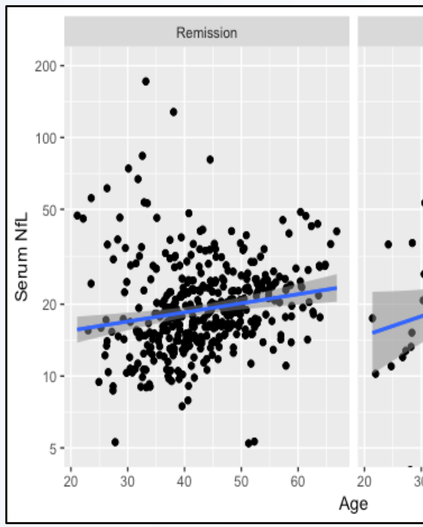
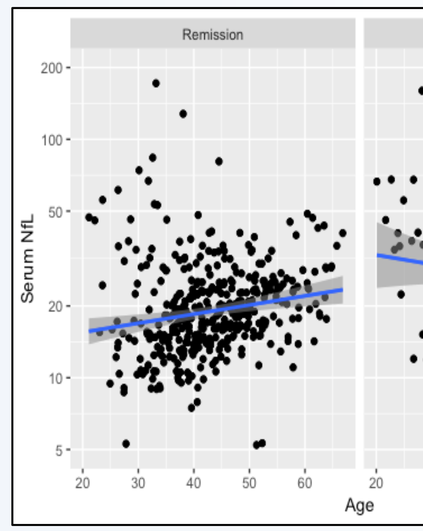


Figure 3. **sNfL dynamics in Remissio**



cytoskeleton and plays an important role for axonal growth and stability
- Serum NfL (sNfL) is a novel biomarker in MS, which has been associated with clinical relapses, gadolinium-enhancing lesions, and brain atrophy
- A few studies have included long follow-up times (e.g. 10-12 years), which further outlines important correlation between baseline axonal damage and long-term axonal loss

OBJECTIVE

- To explore the value of averaged annual serum neurofilament light (sNfL) measures in predicting deep gray matter (DGM) structures like thalamus, caudate, putamen and globus pallidus from a year 10 high-resolution 3T MRI scans

METHODS

SUBJECTS

- We identified 125 patients in the Comprehensive Longitudinal Investigation of MS at Brigham and Women's Hospital (CLIMB) repository who had annual serum samples for up to 10 years [Table1]
- A consistent 3T brain MRI acquisition platform (Siemens Skyra) and protocol was employed to obtain 3D high-resolution FLAIR, T2-weighted, and T1-weighted images, each with 1 mm isotropic voxels. The images were applied to a fully automated pipeline (FSL-FIRST, v. 5.0) to derive volumes of the caudate, putamen, globus pallidus, and thalamus

STATISTICAL ANALYSIS

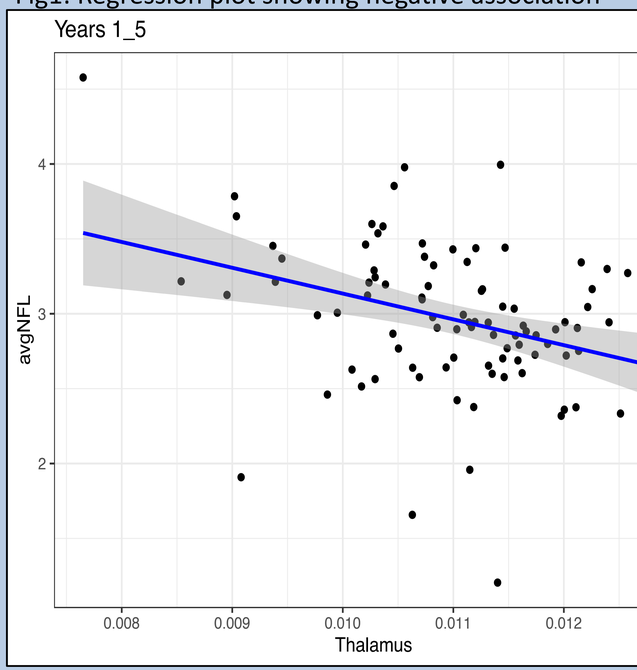
- We ran both univariate and multivariate linear regression analyses to assess the association between baseline averaged annual sNfL levels as the main predictor and 10-year DGM volumes as the outcomes
- The multivariate analyses were adjusted for age, disease duration and sex
- Additionally, we determined the additional variance in DGM explained by sNfL levels by comparing the reduced linear regression model (without sNfL as a predictor) to the full linear regression model (with sNfL as a predictor)
- All analyses were adjusted for multiple testing correction with Holm-Bonferroni correction

Average 1- 2 NFL	79	-1.37e-05	-2.04e-05, -7.01e-06	1.11e-04	1.11e-04	-1.32e-05	-2.01e-05, -6.19e-06	3.38e-04	0.2071	0.0557	4.10
Average 1- 3 NFL	89	-2.24e-05	-3.18e-05, -1.29e-05	9.13e-06	4.28e-05	-2.11e-05	-3.11e-05, -1.12e-05	6.03e-05	0.2275	0.0633	3.62
Average 1- 4 NFL	91	-2.70e-05	-3.90e-05, -1.51e-05	2.03e-05	4.28e-05	-2.54e-05	-3.79e-05, -1.29e-05	1.14e-04	0.2136	0.0639	4.10
Average 1- 5 NFL	91	-2.72e-05	-3.77e-05, -1.67e-05	1.66e-06	1.49e-05	-2.59e-05	-3.73e-05, -1.46e-05	1.82e-05	0.2449	0.0639	1.64
Average 1- 6 NFL	91	-3.09e-05	-4.29e-05, -1.88e-05	2.08e-06	1.66e-05	-2.93e-05	-4.23e-05, -1.62e-05	2.60e-05	0.2388	0.0639	2.08
Average 1- 7 NFL	91	-3.43e-05	-4.81e-05, -2.04e-05	3.97e-06	2.78e-05	-3.25e-05	-4.75e-05, -1.75e-05	4.49e-05	0.2296	0.0639	3.14
Average 1- 8 NFL	91	-3.67e-05	-5.20e-05, -2.13e-05	8.21e-06	4.28e-05	-3.42e-05	-5.08e-05, -1.76e-05	9.10e-05	0.2174	0.0639	4.10
Average 1- 9 NFL	91	-3.53e-05	-4.99e-05, -2.06e-05	7.14e-06	4.28e-05	-3.28e-05	-4.86e-05, -1.71e-05	8.19e-05	0.2192	0.0639	4.10
Average 1-10 NFL	91	-3.47e-05	-4.97e-05, -1.98e-05	1.28e-05	4.28e-05	-3.20e-05	-4.80e-05, -1.60e-05	1.44e-04	0.2094	0.0639	4.10

Table1: Demographics

Characteristic	Feature	N
Patients with MS		122
Race (N, %)	Black	2 (1.64%)
	Missing	1 (0.82%)
	More than one race	1 (0.82%)
	Unknown/not reported	1 (0.82%)
	White	117 (95.80%)
Sex (N, %)	Female	89 (72.95%)
	Male	33 (27.05%)
Age at the first sample (mean, SD)		37.95 ± 9.09
Age at first symptom (mean, SD)		36.35 ± 9.01
Disease duration at first visit (mean, SD)		1.61 ± 1.08

Fig1: Regression plot showing negative association



DISCLOSURES

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Multivariate Protein Biomarker Models Better Predict Multiple Sclerosis MRI Disease Activity Compared to Serum Levels of Neurofilament Light Chain Alone

Tanuja, Chitnis[1] Hajime Yano[1], Shrishti Saxena[1], Hrishikesh Lokhande[1], Neda Sattarnezhad[1], Anu Paul[1], Fermisk Saleh[1], Mikaela Collins[1], Bonnie Glanz[1], Charles Guttmann[1], Rob Bakshi[1], Ferhan Qureshi[2], Michael Becich[2], Victor Gehman[2], David Hughes[2], Howard Weiner[1]

[1]Partners MS Center, Ann Romney Center for Neurologic Diseases, Department of Neurology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA; [2] Octave Bioscience, Menlo Park, CA, USA

INTRODUCTION

BACKGROUND:

- Multiple sclerosis(MS) is a chronic inflammatory demyelinating disease of the central nervous system with various phenotypes and heterogenous disease course. [1]
- While exact pathophysiology of MS remains elusive, both inflammatory and degenerative processes are believed to play a role in the disease mechanism and disability progression. [1,2]
- Identifying disease-specific biomarkers may assist with predicting the diverse disease course and classifying patients to high risk versus low risk for disease activity and progression. [3,4]
- Use of multivariate models reflecting multiple biological pathways that are involved in the complex pathophysiology of MS will most likely increase predictive accuracy of these biomarkers. [4]
- Serum levels of neurofilament light chain (sNfL) are associated with neurodegeneration in Multiple Sclerosis (MS) and correlate with measurements of disease activity (DA), including the presence of gadolinium enhancing (GAD+) lesions.
- The inclusion of additional inflammatory and neurodegenerative protein biomarkers, can provide deeper insights and reveal stronger correlations to radiographic DA than sNfL individually.

OBJECTIVES:

To compare the performance of multivariate protein biomarker models with sNfL individually to classify samples from subjects with and without GAD+ lesions from the Comprehensive Longitudinal Investigation of Multiple Sclerosis at Brigham and Women's Hospital (CLIMB) study.

METHODS and MATERIALS

SUBJECTS: A total of 326 serum samples drawn within close-proximity (median interval 1 day) to a contrast-enhanced MRI were measured for 1196 proteins including sNfL using Proximity Extension Assays (PEA) from Olink and 215 proteins using Luminex based immunoassays from Rules Based Medicine (RBM). Samples represented both 113 longitudinal pairs (n=226) and non-paired specimens (n=100) that were categorized by the number of GAD+ lesions per Table 1. 58 samples had been measured previously for 1104 proteins using the Olink platform as a proof of concept study.

Table 1: GAD lesion count distribution for paired and unpaired samples

Sample Group (# of GAD+ lesions)	Sample Pairs	Individual Samples	0 lesions	1 lesion	2 lesions	≥3 lesions
A (0 and ≥1)	98	196	98	66	19	13
B (1 and ≥2)	15	30	0	15	7	8
C (≥ 2)	0	100	0	0	77	23
Totals	113	326	98	81	103	44

STATISTICAL ANALYSIS: Univariate and multivariate machine learning-driven biostatistical techniques were used to classify samples with and without GAD+ lesions. Analysis was performed both on the entire cohort (n=326) and restricted to longitudinal pairs which strictly included a sample with 0 GAD+ lesions. Five-fold cross-validation and regularization (L2) were used in tandem with sequential feature selection to minimize overfitting and ensure generalizability for predicting DA of new samples. Area Under the Curve (AUC) and Accuracy were selected as the key metrics for comparison.

RESULTS-I

FEATURE SELECTION: Exploratory data analysis was conducted to filter noise, reduce dimensionality & avoid collinearity. Univariate significance was combined with multivariate importance from simulated models as shown in Table 2.

P-value	AUC	Protein
6.06E-15	0.746	sNfL
0.015	0.585	CNTN5
0.046	0.552	E-Cad
0.006	0.575	TNC
0.030	0.577	SORT1
0.070	0.574	PDGFC
0.009	0.573	CTSF
0.006	0.582	TNFRSF10A
0.023	0.568	VWA1
0.014	0.569	IL18
2.55E-04	0.62	CCL20
1.39E-04	0.622	CDCP1
2.50E-04	0.627	CXCL9
0.019	0.572	CCL3
0.004	0.588	PODXL2
0.004	0.582	EZR
0.028	0.568	LIF-R
0.082	0.567	GDF-15
0.040	0.556	MFGE8
0.012	0.569	CXCL6
0.020	0.566	YKL-40

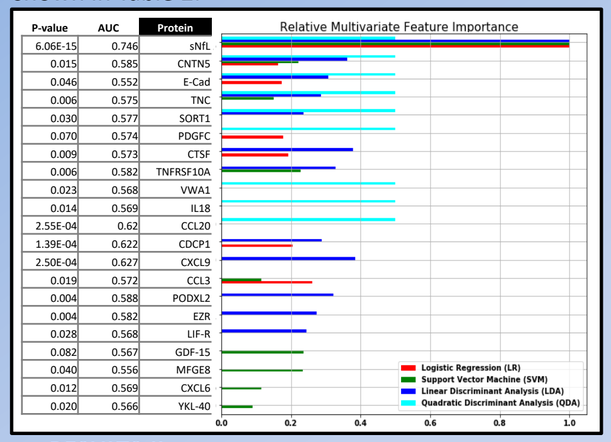

Table 2: Top 21 features ranked by feature importance (across LR, SVM, LDA, and QDA models), shown by accompanying p-value (2 sample, 1-sided homoscedastic t-test) and univariate AUC (trapezoidal integration of TPR, FPR across all 326 samples). sNfL passed multiple hypothesis correction filters (Bonferroni) for paired and unpaired samples while the remaining markers contribute orthogonal signal that were deemed significant explanatory variables (nonzero) through 95% confidence intervals after 100,000 bootstrap iterations. A similar procedure was conducted for the 196 paired samples to identify the strongest shifts (not shown).

RESULTS-II

MODEL-BUILDING: Forward selection, combined with grid search hyperparameter-tuning, as measured by 5-fold stratified cross-validation, achieved strong separation potential across supervised classification models: AUC_{LR} = 0.836 ± 0.066, AUC_{SVM} = 0.834 ± 0.039, AUC_{QDA} = 0.827 ± 0.055, AUC_{LDA} = 0.822 ± 0.065. The highest-performing parsimonious model (a 7- feature logistic regression model) was then validated using 100,000 iterations of repeated 50/50 cross-validation to produce the ROC curves in Fig. 1.

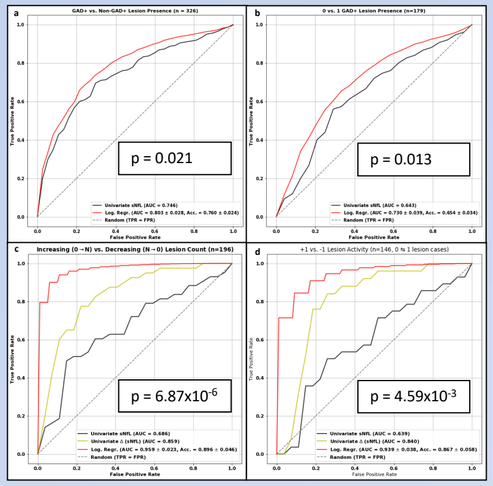

Figure 1. The Receiver Operating Characteristic (ROC) curve visualizes the true and false positive rates of various thresholds to separate the protein levels across samples. The p-value represents the statistical significance of the multivariate model's AUC being significantly greater than the AUC of sNfL (or Δ sNfL). ROC plots b and d reflect the power of the model to discriminate 0 vs. 1 lesions (thereby representing subtle disease activity). Different features were pulled in for the longitudinal analysis; however, in all 4 breakdowns of the study (a-d), logistic regression models showed significantly ($p < 0.05$) improved sensitivity and specificity (as measured by AUC).

Biomarkers that were selected as important features in the multivariate classifier were investigated for relevance and interactions using biological network models. In addition to neurodegeneration, proteins related to inflammatory and immune pathways were identified.

CONCLUSIONS

- Multivariate protein biomarker models representing several biological pathways predicted radiographic DA with greater statistical significance than sNfL alone.
- A multivariate model based on shifts in patient protein levels (between 2 samples, which better controls for age/sex/BMI) was able to strongly predict directionality of lesion activity (AUC=0.96). This not only outperforms sNfL alone, but also improves upon the multivariate model's ability to predict predict lesion presence from an individual MS patient's blood sample (AUC=0.80).
- Further investigation with larger sample numbers and from additional cohorts is warranted.

REFERENCES

1)Wingerchuk, Dean M., and Jonathan L. Carter. "Multiple sclerosis: current and emerging disease-modifying therapies and treatment strategies." *Mayo Clinic Proceedings*. Vol. 89. No. 2. Elsevier, 2014. 2) Macaron, Gabrielle, and Daniel Ontaneda. "Diagnosis and Management of Progressive Multiple Sclerosis." *Biomedicines* 7.3 (2019): 56. 3) Comabella, Manuel, and Xavier Montalban. "Body fluid biomarkers in multiple sclerosis." *The Lancet Neurology* 13.1 (2014): 113-126.4) Barro, Christian, et al. "Serum neurofilament as a predictor of disease worsening and brain and spinal cord atrophy in multiple sclerosis." *Brain* 141.8 (2018): 2382-2391.

DISCLOSURES

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Contact email address: tchitnis@rics.bwh.harvard.edu