P0055

Cross-sectional and longitudinal estimation of radiographic and clinical endpoints to quantify MS disease trajectory with blood serum protein levels

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Disclosures

- **Jens Kuhle received speaker fees, research support, travel support, and/or served on advisory boards by ECTRIMS, Progressive MS Alliance, Swiss MS Society, Swiss National Research Foundation (320030_189140/1), University of Basel, Bayer, Biogen, Celgene, Merck, Novartis, Roche, Sanofi.**
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- **D. Leppert is CMO of GeNeuro; he has received personal compensation for consulting and speaking, and travel reimbursement from Quanterix, Roche, Novartis, Orion, GeNeuro and Sanofi.**
- **C. Granziera: The University Hospital Basel (USB), as the employer of Cristina Granziera has received the following fees which were used exclusively for research support: (i) advisory board and consultancy fees from Actelion, Novartis, Genzyme and F. Hoffmann-La Roche; (ii) speaker fees from Biogen and Genzyme-Sanofi; (iii) research support by F. Hoffmann-La Roche Ltd. Before my employment at USB, I have also received speaker honoraria and travel funding by Novartis.**
- **Michael Becich, Victor Gehman, Amal Katrib, Fatima Rubio da Costa and Ferhan Qureshi are employees of Octave Bioscience.**

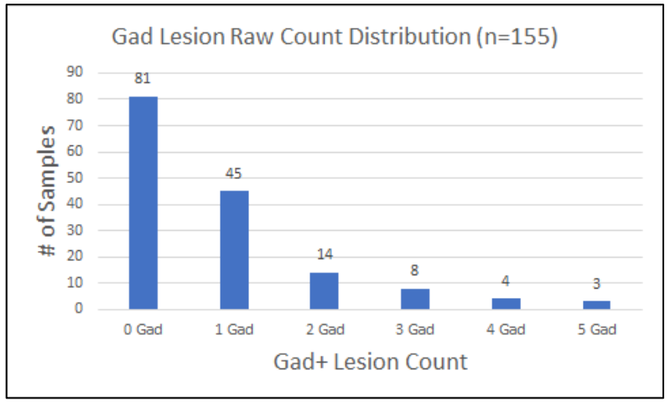
Background and Objectives

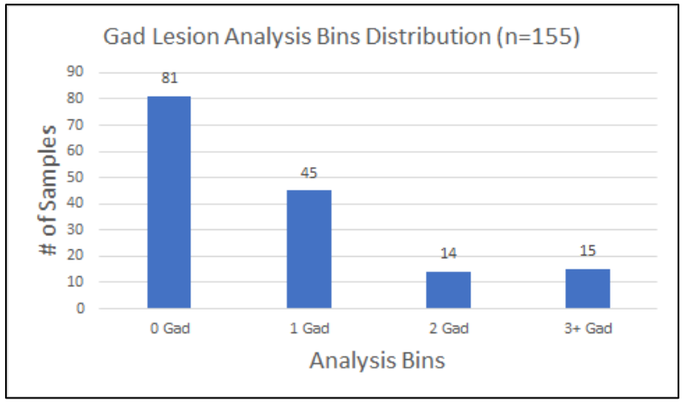
- Quantification of Disease Activity (DA) and Disease Progression (DP) are important tools for MS research and can also be utilized to enhance clinical treatment.
- DA and DP assessments currently rely on qualitative clinical evaluations or the acquisition of magnetic resonance imaging (MRI).
- Quantifying DA and DP instead through the use of blood biomarkers would provide a significant reduction to several barriers in such assessments:
  - Cost, time, evaluation subjectivity, invasiveness, and operational difficulty.
- Association with several MS endpoints (Gad lesions, ARR, and clinical relapse status) in earlier feasibility studies alongside computational biology modeling led to the development of a custom 21-plex proteomic assay panel.
- **Objectives:** To analyze the expression levels of these 21 proteins relative to clinical and radiographic endpoints in a cohort of samples from the University Hospital Basel.

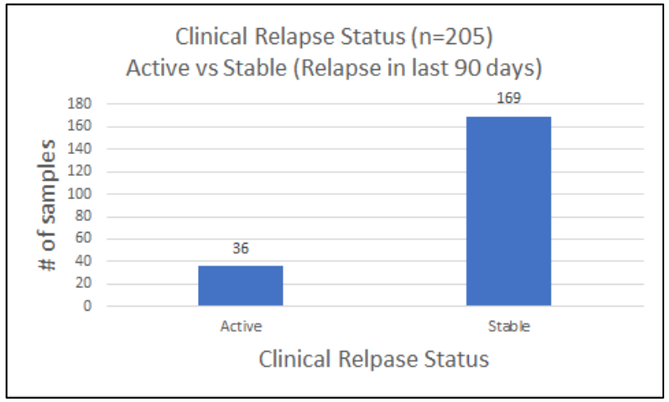
Cohort Characteristics

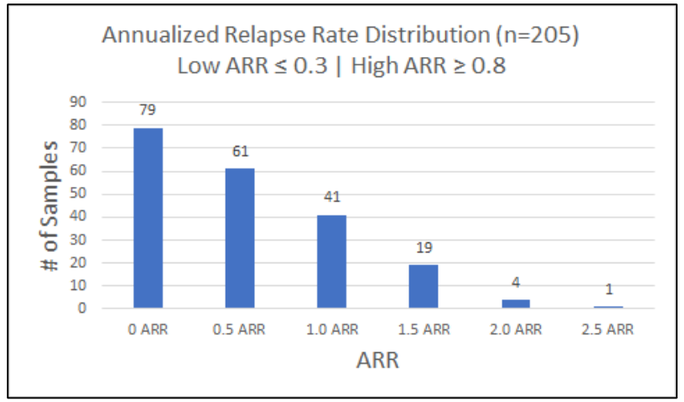
- n=205 serum samples from University Hospital Basel
- Biobanking dates range from July 2012 to August 2019
- 88 subjects (67 female, 21 male) - all longitudinal
  - 2 timepoints = 73
  - 3 timepoints = 3
  - 4 timepoints = 11
  - 6 timepoints = 1
- MRI analysis: Gad lesions were assessed via manual expert detection. T2 lesions were assessed via deep learning based detection with manual correction
- **Primary Endpoint**: Gadolinium (Gad) enhanced lesion count*
- **Secondary Endpoints**: T2 lesion volume*, Expanded Disability Status Scale (EDSS) score, Clinically Defined Relapse Status, and Annualized Relapse Rate (ARR)

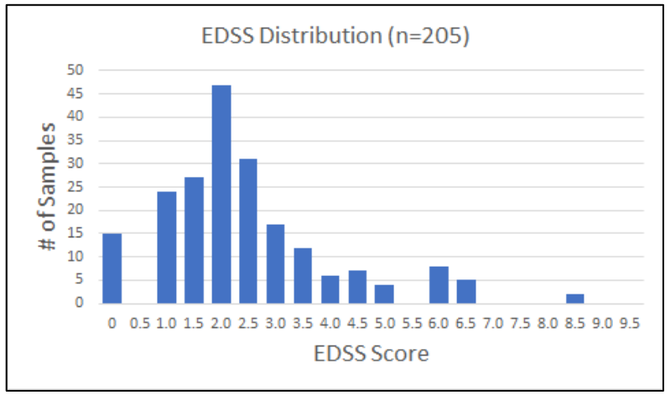
	Gad+ Samples	Non-Gad+ Samples	Total (All Samples)
Age	39.8 ± 11.4 y	41.9 ± 11.7 y	40.8 ± 11.6 y
MS Disease Duration	11.4 ± 10.0 y	12.4 ± 10.9 y	11.9 ± 10.5 y
% Female	78% (25)	76% (22)	77% (47)
EDSS	2.4 ± 1.6	2.5 ± 1.6	2.4 ± 1.6
% within 30 days of MRI	69.8% (74)	81.9% (81)	75.6% (155)
Sample Count	106	99	205

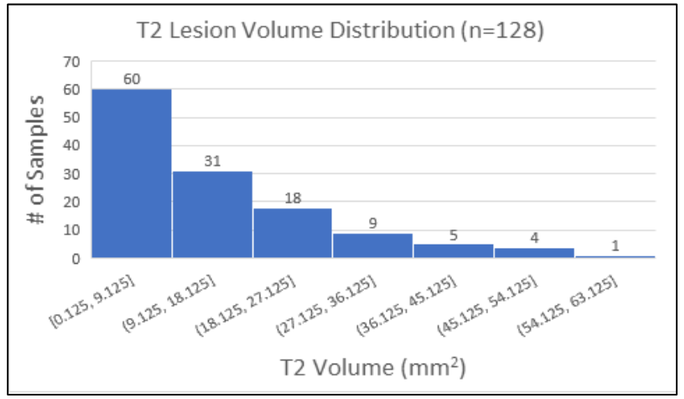










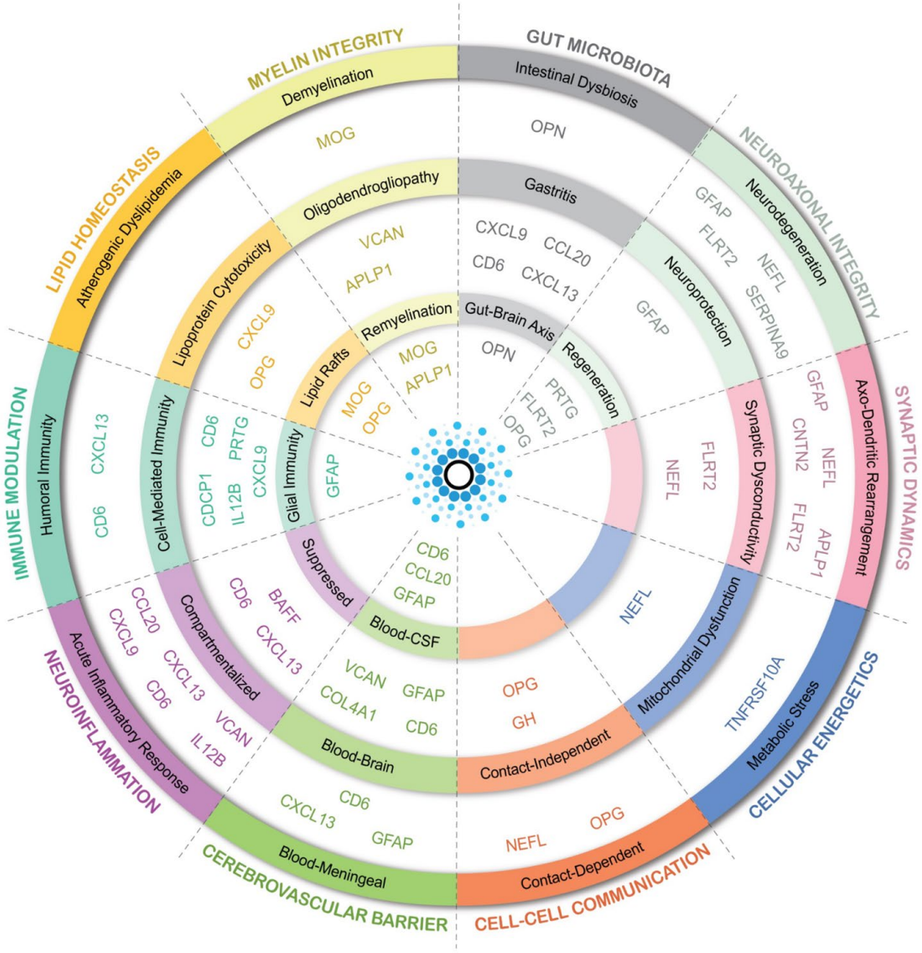


*For radiographic endpoints, 30 day threshold was applied for timing between MRI and blood draw.

Analytical Methods

- 205 MS serum samples were analyzed for relative expression levels of 828 proteins on the Olink™ platform.
- Results flagged with analytical quality control warnings were removed from the statistical analysis.
- Results below limits of detection (LOD) were imputed to the assay specific LOD values.
- A focused statistical analysis relative to several radiographic and clinical endpoints was conducted for 20 proteins.
- Samples were then re-analyzed in Custom Assay Panel (21 proteins) and reported in absolute concentration units (pg/mL).
  - Results from an additional analyte (GFAP) were available in the dataset generated using the Custom Assay Panel.

Biomarkers in Custom Assay Panel - Association with MS Hallmarks



Proximity Extension Assay Methodology

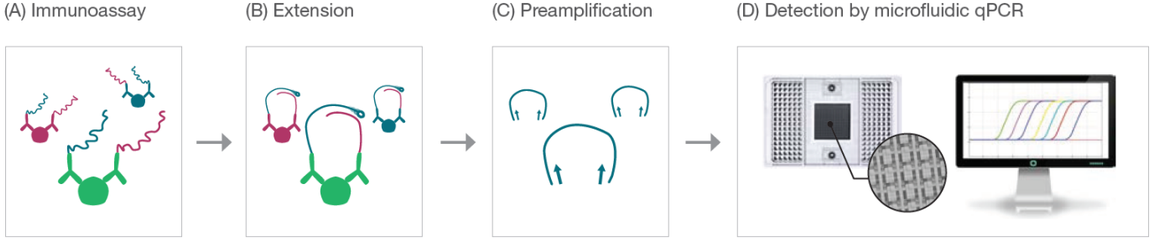
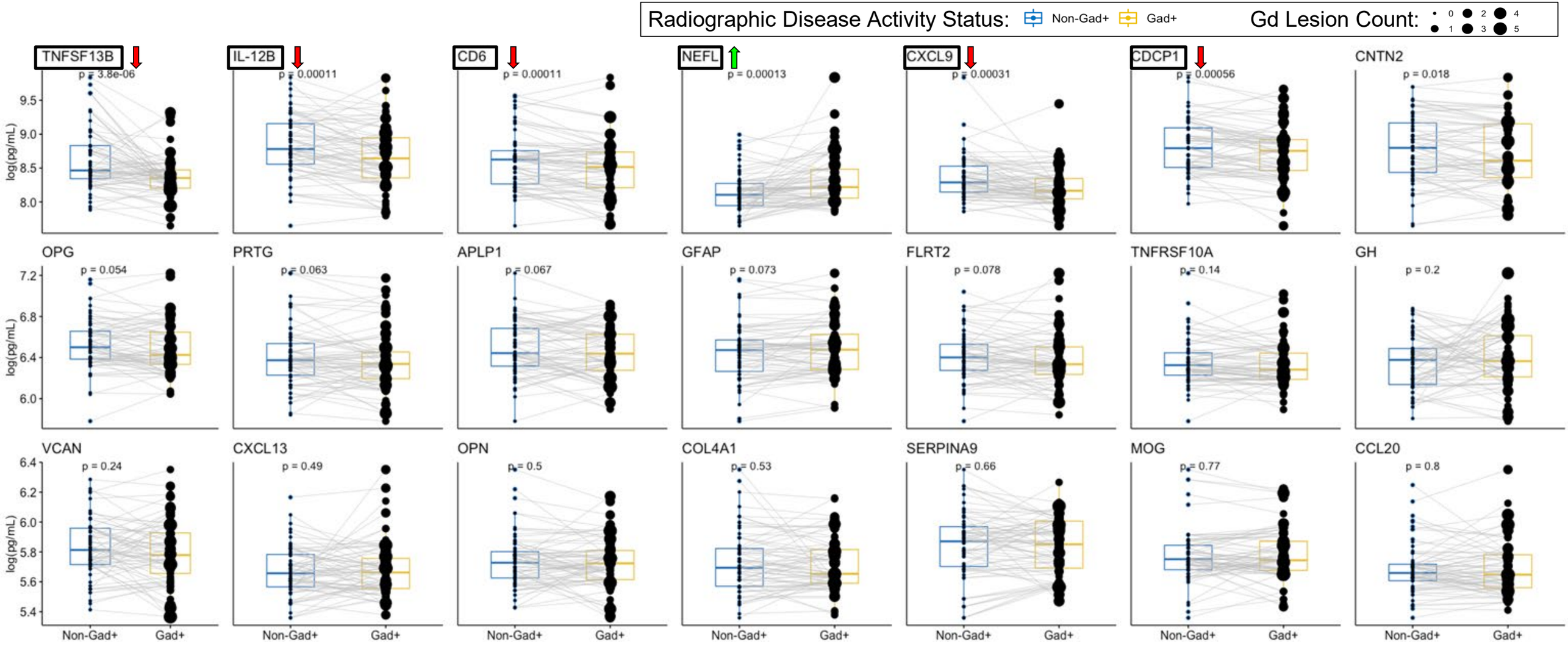


Fig 1. Overview of the PEA technology. (A) 92 Antibody pairs, labelled with DNA oligonucleotides, bind target antigen in solution. (B) Oligonucleotides that are brought into proximity hybridize, and are extended by a DNA polymerase. (C) This newly created piece of DNA barcode is amplified by PCR. (D) The amount of each DNA barcode is quantified by microfluidic qPCR.

Primary Endpoint Univariate Results

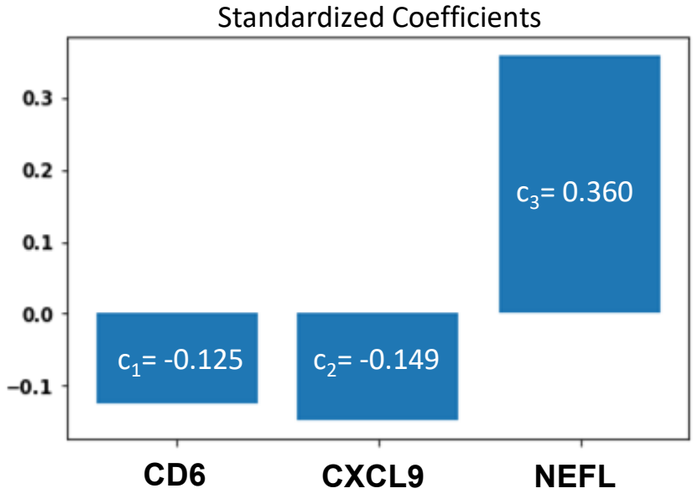
- Dots over box-and-whisker plots represent Gd lesion count per sample
  - Linking shows movement in biomarker concentration between a Non-Gad+ (0 lesion) and Gad+ (1+ lesions) pair from the same patient.
  - n=155 samples where serum draw and MRI fell within 30 days of one another
- Sorted by statistical significance (paired t-test)
  - Biomarkers labeled with box are significant at FDR=0.05 with Benjamini-Hochberg procedure for multiple hypothesis correction with indicated directionality ⇑⇓
- No demographic adjustment applied since baseline sample difference accounts for inter-patient variation.



Multivariate Analysis - Gd Lesions

- **Baseline Normalization:** Longitudinal biomarker shifts from a Non-Gad+ baseline
- Classifying patient longitudinal sample pairs with increasing (Gd- → Gd+) vs. decreasing (Gd+ → Gd-) Lesion Burden
- Univariate features demonstrate paired significance at Benjamini-Hochberg MH Correction (FDR = 0.05)
- Independent Holdout Model Performance Evaluation
  - Train on CLIMB Cohort (n=186)
  - Test on Basel Cohort (n=146)
- Logistic Regression Model (Weighted Sum of Coefficients):

$$c_1 \triangle log[sCD6] + c_2 \triangle log[sCXCL9] + c_3 \triangle log[sNfL] \sim \triangle GdLesions$$



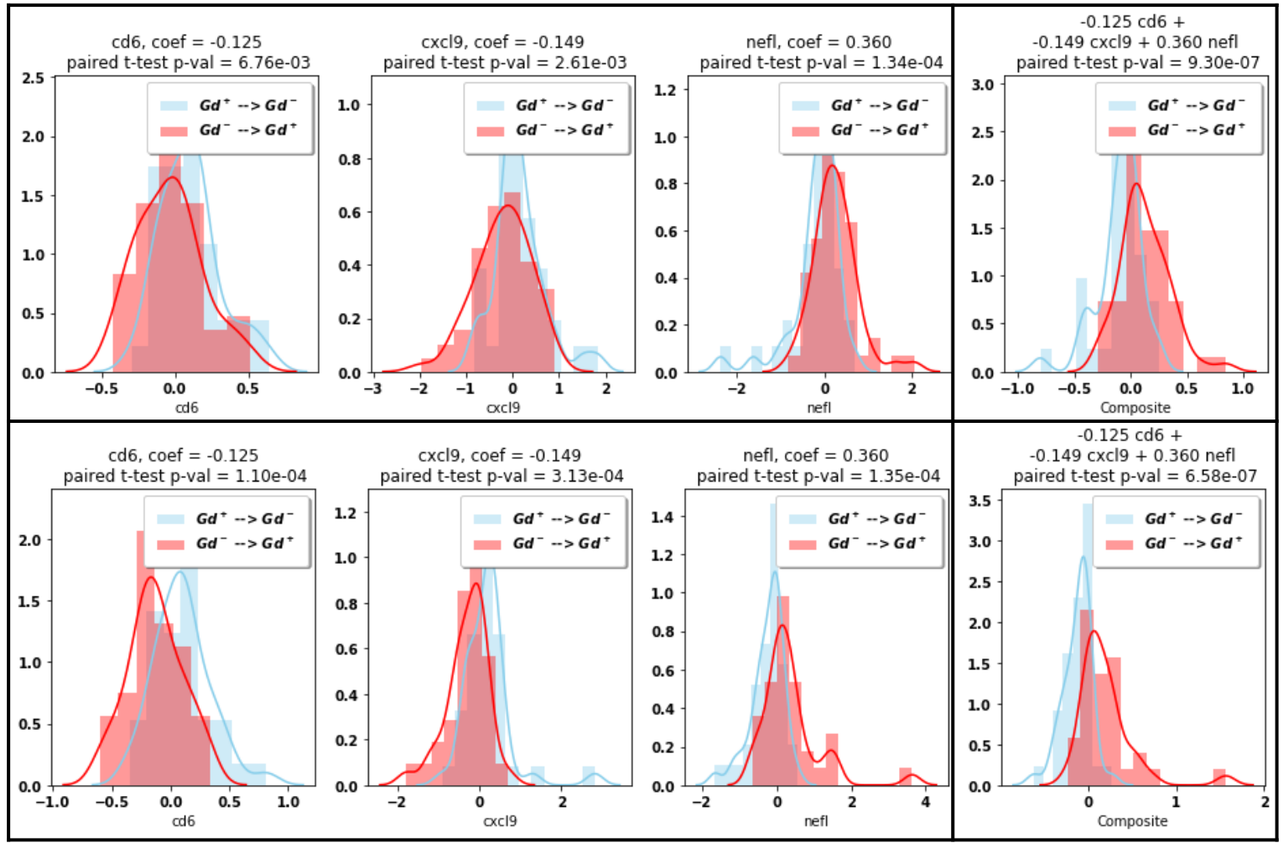
AUROC: 0.92 (sNfL - 0.77)
Accuracy: 0.85 (sNfL - 0.73)
Sensitivity: 0.85 (sNfL - 0.74)
Specificity: 0.85 (sNfL – 0.71)
Youden's Statistic = 0.70 (sNfL – 0.45)

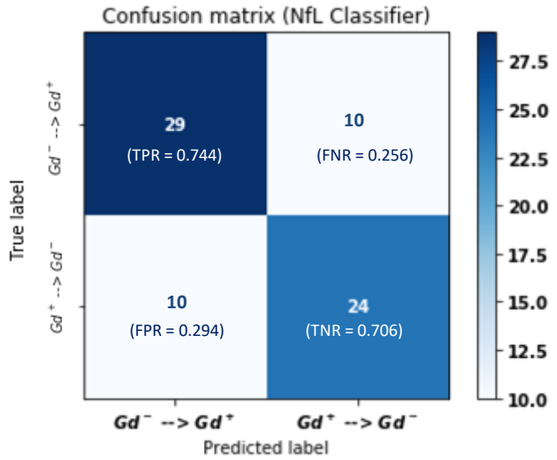
Individual Proteins

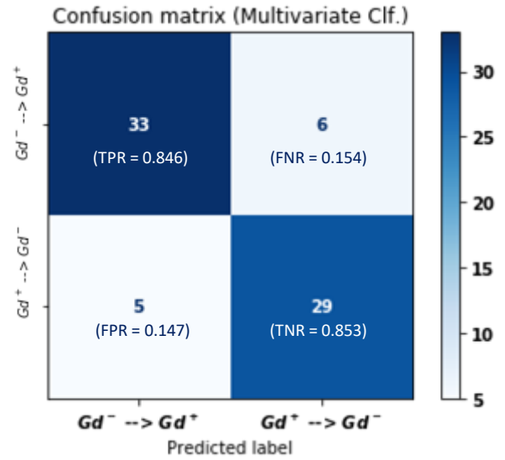
Multivariate Model

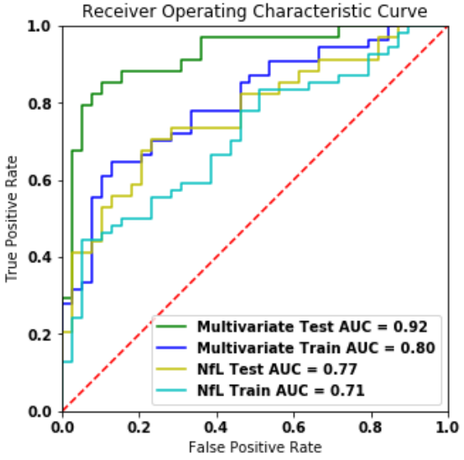
Train (93 pairs)

Test (73 pairs)









Primary, Secondary Endpoint Univariate Results

- Biomarkers from custom assay panel sorted alphabetically.
- Serum samples drawn greater than 30 days outside of an MRI were excluded from Gd and T2 analysis
- Annualized Relapse Rate (ARR) divided into low (≤0.3) and high (≥0.8) groups
- Statistics from unadjusted data shown. Demographic adjustment applied to each biomarker level in cross-sectional multivariate models with respect to Age, Disease Duration, and Sex.
- Highest significance association for each marker **bolded**.

Protein Biomarker	Gad presence p-value (n=155)	EDSS $R2$ (n=205)	Relapse p-value (n=205)	ARR p-value (n=144)	T2-weighted Volume $R2$ (n=128)
APLP1	0.228	3.40E-04	0.084	0.937	0.012
CCL20	0.326	0.038	0.107	0.609	0.038
CD6	0.012	0.001	0.463	0.511	0.001
CDCP1	0.010	0.087	0.306	0.046	2.22E-03
CNTN2	0.050	0.006	0.514	0.141	0.022
COL4A1	0.248	0.006	0.250	0.208	0.007
CXCL13	0.699	0.008	0.093	0.750	4.36E-04
CXCL9	0.010	0.017	0.028	**0.001**	0.026
FLRT2	0.292	0.003	0.435	0.825	0.007
GFAP	0.582	**0.161**	0.221	0.453	**0.122**
GH	0.197	1.30E-03	0.665	0.381	0.019
IL-12B	0.007	0.007	0.068	0.238	0.014
MOG	0.646	4.03E-04	0.062	0.599	0.025
NEFL	0.001	0.090	**2.50E-05**	0.042	0.125
OPG	0.652	0.152	0.612	0.634	0.044
OPN	0.178	0.046	0.086	0.436	0.044
PRTG	0.236	0.004	0.400	0.272	2.22E-03
SERPINA9	0.081	0.010	0.936	0.076	8.75E-04
TNFRSF10A	0.348	0.033	0.984	0.012	0.016
TNFSF13B	**7.63E-05**	0.038	0.335	0.816	0.018
VCAN	0.198	0.043	0.045	0.911	0.018

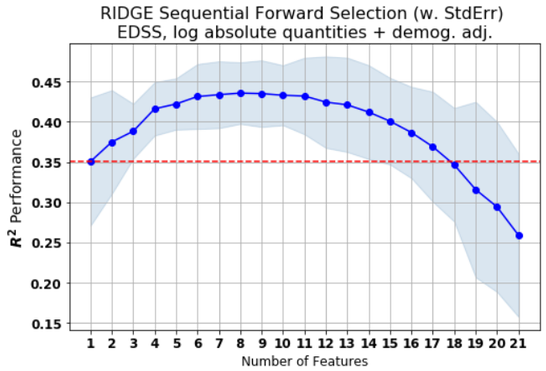
*p-value ≤ 0.05 highlighted in green. R^2 values are visualized on a gradient from 0 to the highest reported value.

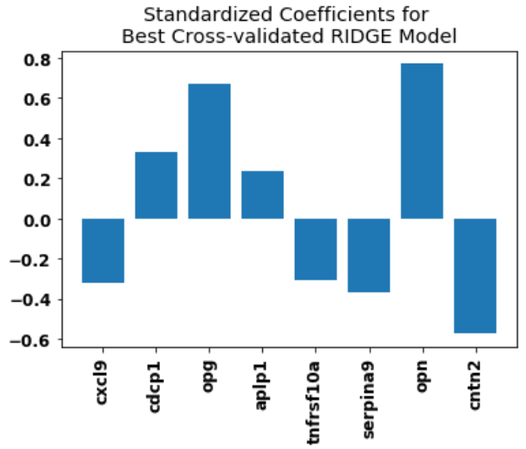
Secondary Endpoints Multivariate Results

Forward selection analysis: 5-fold Cross Validation. Reported values are mean ± standard deviation.

EDSS

(n=205)

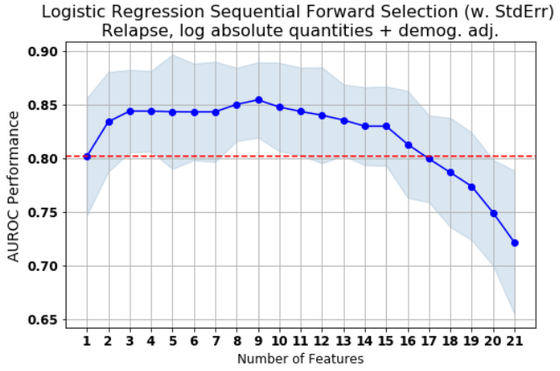


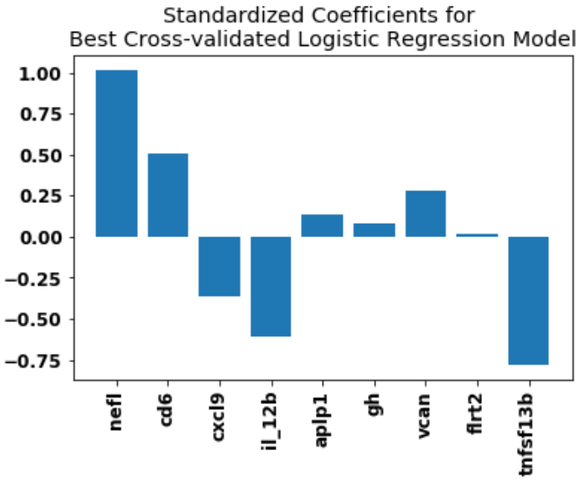


OPN R^2: 0.376 ± 0.036
Multivariate R^2: 0.446 ± 0.038

Relapse Status

(n=205; 169 neg / 36 pos)

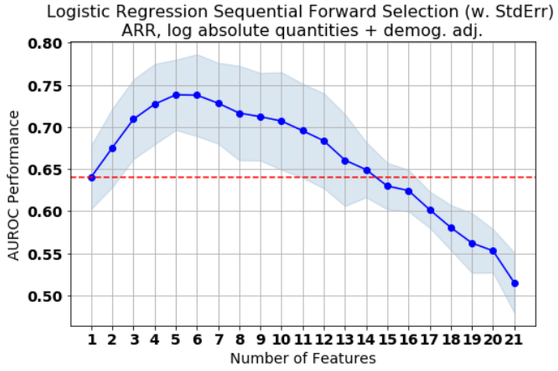


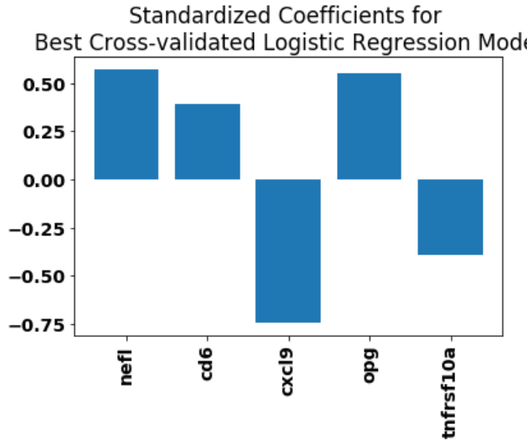


NEFL AUC: 0.801 ± 0.123
Multivariate AUC: 0.854 ± 0.079

ARR

(n=112; 70 low / 42 high)

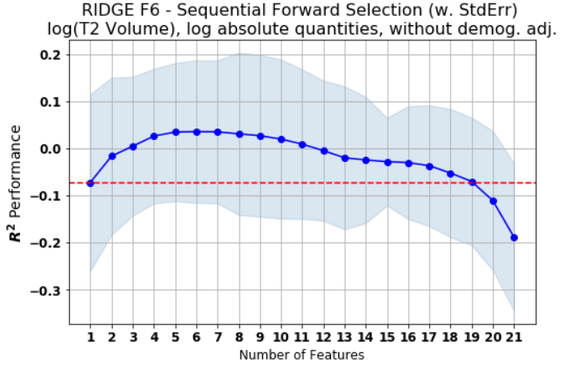


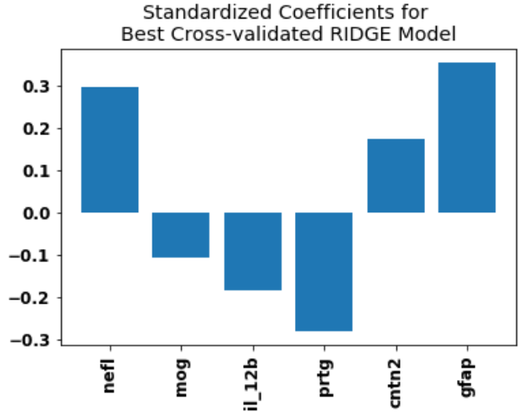


NEFL AUC: 0.640 ± 0.084
Multivariate AUC: 0.738 ± 0.093

T2 Volume

(n=128)





GFAP R^2: 0.105 ± 0.030
Multivariate R^2: 0.147 ± 0.041

R^2 values were estimated fitting a linear regression model to the measured endpoint vs. the estimated value.
AUC values were estimated using a logistic regression classification algorithm.

Conclusions / Discussion

- Statistical results from primary endpoint of Gd lesion count (performance on independent test set):
  - AUROC: sNfL+sCD6+sCXCL9 (0.92) > sNfL (0.77)
  - Accuracy: sNfL+sCD6+sCXCL9 (0.85) > sNfL (0.73)
  - Sensitivity: sNfL+sCD6+sCXCL9 (0.85) > sNfL (0.74)
  - Specificity: sNfL+sCD6+sCXCL9 (0.85) > sNfL (0.71)
    - **Baseline normalization reveals replicating 3-protein signature across independent cohorts (CLIMB, Basel)**
- Statistical results for secondary endpoint (multivariate cross-validated performance, p-value relative to best univariate model):
  - EDSS: mild association (R^2 = 0.45, p = 0.001)
  - Clinically-Defined Relapse: strong classifier (AUC = 0.85, p = 0.21)
  - ARR: moderately strong classifier (AUC = 0.74, p = 0.04)
  - T2-weighted lesion volume (log-transformed): weak association (R^2 = 0.15)
- **Next Steps:**
  - Investigate protein signatures in sample pairs with shifting Gd counts vs. non-shifting Gd count.
  - Longitudinal MRI images are being processed using an internal quantitative imaging pipeline for analysis relative to serum biomarkers.
  - Analytical Validation studies (Accuracy, Precision, Sensitivity, Specificity, etc.) for the 21 plex Custom Assay Panel are ongoing.
  - Clinical Validation studies for Custom Assay Panel are upcoming: Association with DA endpoints in independent cohorts.
  - Proposed Clinical Utility for a validated DA Test: (1) Identification of active relapse (2) Prediction of impending relapse (3) Confirmation of NEDA status (4) Assessment of longitudinal changes relative to previous tests and (5) Response to DMTs

Questions? Please Contact - Ferhan Qureshi: fqureshi@octavebio.com - Dr. Jens Kuhle: jens.kuhle@usb.ch

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