P0063

Development of a Custom Multivariate Proteomic Serum Based Assay for Association with Radiographic and Clinical Endpoints in MS

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Introduction

- Multiple Sclerosis (MS) is a complex and heterogeneous disease.
- Protein biomarker expression can inform the development of tools to:
  - Monitor Disease Activity
  - Monitor Disease Progression
  - Identify early evidence of relapse
  - Monitor treatment response
- **Objective**: To develop a blood based multiplex proteomic assay that associates with clinical and radiographic endpoints in patients with MS.
  - These endpoints include the presence of gadolinium-enhanced (Gd+) lesions, Annualized Relapse Rate (ARR) and clinically defined relapse status (active versus stable).

Disclosures:

Tanuja Chitnis has received research funding from Octave Biosciences.

Michael Becich, Victor Gehman, Amal Katrib, Fatima Rubio da Costa and Ferhan Qureshi are employees of Octave Bioscience.

Cohort Characteristics

Serum samples from three deeply phenotyped cohorts were analyzed for protein levels and associated with clinical and radiographic endpoints to select features for inclusion in the custom assay panel.

ACP[1]	Exacerbation Samples	Quiessence Samples	Total (All Samples)
Age	38.7 ± 10.1 y	35.5 ± 9.3 y	38.8 ± 9.6 y
MS Disease Duration	1.2 ± 2.3 y	3.8 ± 2.1 y	2.5 ± 2.5 y
% Female	77% (46)	73% (47)	75% (93)
Count	60	64	124

EPIC[3]	Gad+ Samples	Non-Gad+ Samples	Total (All Samples)
Age	40.3 ± 9.1 y	43.9 ±10.4 y	41.2 ± 9.5 y
MS Disease Duration	9.4 ± 8.9 y	11.6 ± 8.8 y	9.9 ± 8.9 y
% Female	74% (100)	71% (32)	73% (132)
Count	135	45	180

CLIMB[2]	Gad+ Samples	Non-Gad+ Samples	Total (All Samples)	LOW (≤0.2) ARR	HIGH (≥0.8) ARR
Age	38.1 ± 9.4y	40.5 ± 8.0y	38.8 ± 9.1y	39.7 ± 9.6y	31.8 ± 7.5y
MS Disease Duration	7.3 ± 6.1y	8.5 ± 6.4y	7.7 ± 6.2y	8.7 ± 7.1y	2.0 ± 2.2y
% Female	74% (168)	74% (73)	73% (242)	73% (108)	65% (13)
Sample Count	228	98	326	148	20

ACP Endpoint:

Primary: Clinically Defined Relapse Status - Exacerbation vs Quiessence

CLIMB Endpoints:

Primary: Radiographically Defined Relapse Status - Gad Lesions

Secondary: Annualized Relapse Rate

EPIC Endpoint:

Primary: Radiographically Defined Relapse Status - Gad Lesions

[1] Accelerated Cure Project
[2] Comprehensive Longitudinal Investigation of MS at Brigham and Women's Hospital
[3] Expression, Proteomics, Imaging, Clinical at UCSF

Analytical Methodology

Proximity Extension Assay Methodology

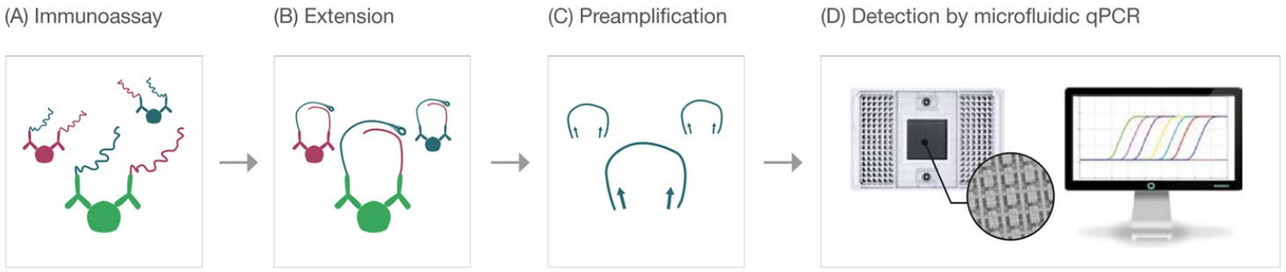
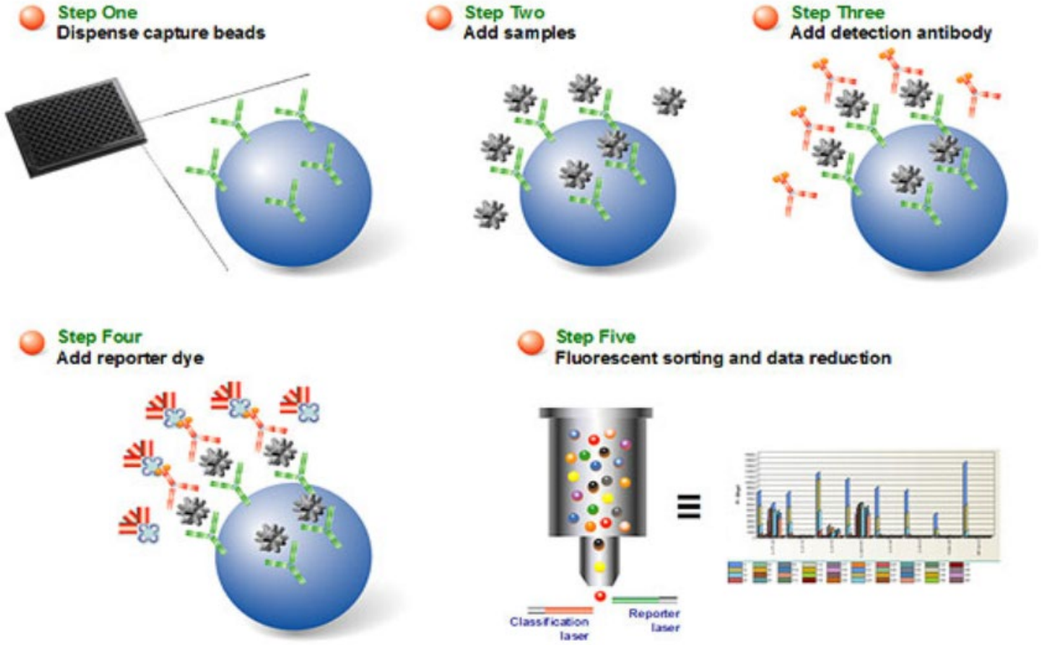


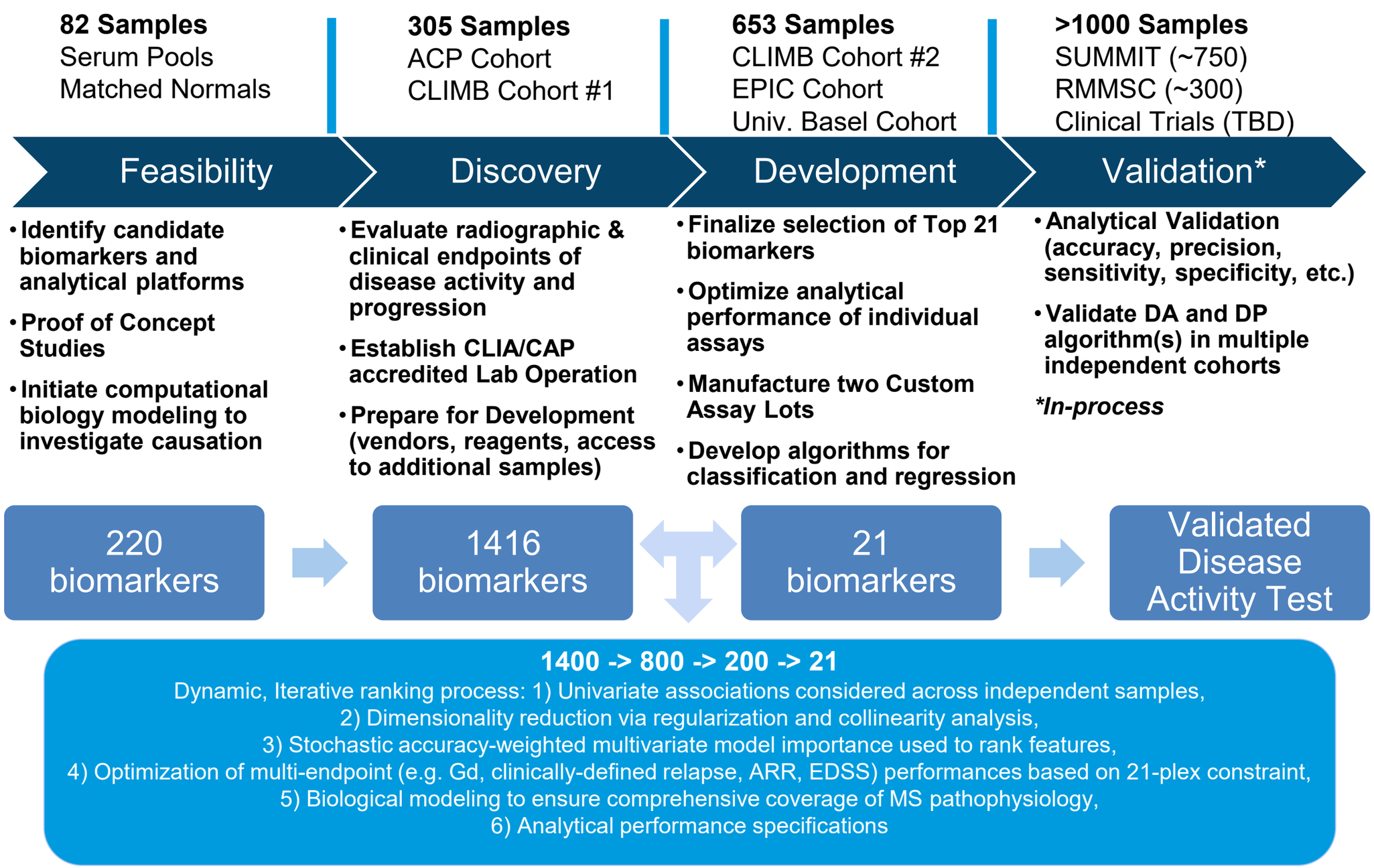
Fig 1. Overview of the PEA technology. (A) 92 Antibody pairs, labelled with DNA oligonucleotides, bind target antigen in solution. (B) Oligonucleotides that are brought into proximity hybridize, and are extended by a DNA polymerase. (C) This newly created piece of DNA barcode is amplified by PCR. (D)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

Luminex xMAP Assay Methodology



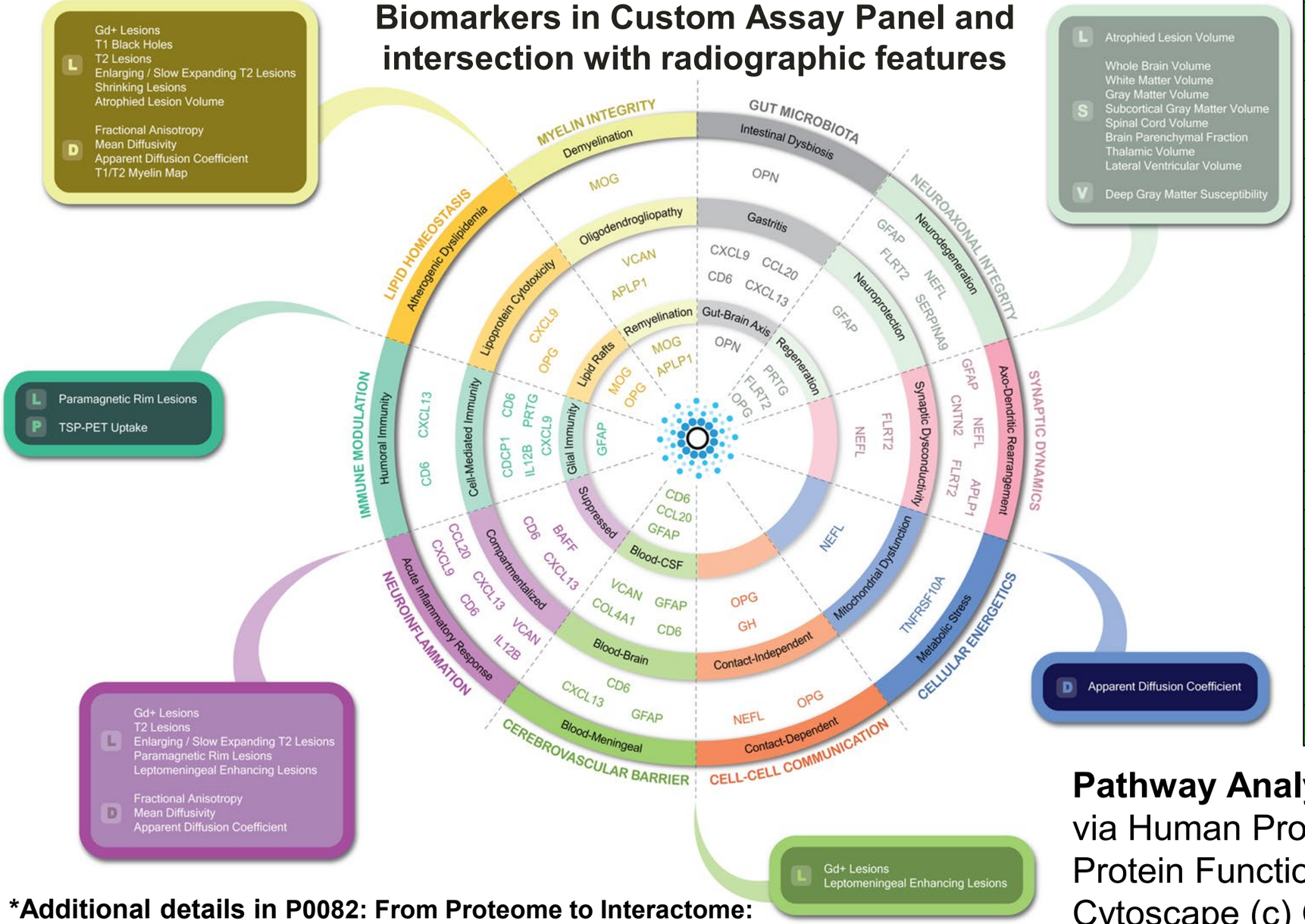
- ACP and CLIMB serum samples were analyzed to determine the concentration of 215 proteins using Luminex based xMAP® technology immunoassays at Myriad RBM, Inc.
- ACP, CLIMB and EPIC serum samples were analyzed to determine the relative expression levels for up to 1196 proteins using Proximity Extension Assays on the Olink™ platform.
- Results that were flagged with analytical QC warnings were either rerun or removed from the statistical analysis.
- Results that were below the individual assays limit of detection (LOD) or limit of quantitation (LOQ) were imputed to the assay specific LOD/LOQ value.
- 21 Biomarkers were selected to include in a single custom assay panel on the Olink™ Platform based on their univariate and multivariate associations with clinical and radiographic MS endpoints.
- Biological pathway modeling and network analysis were performed to ensure comprehensive representation of MS neurophysiology.
- The custom assay panel has been manufactured to include calibrators in order to report results in absolute concentration and is undergoing a fit-for-purpose analytical validation.

Protein Biomarker Selection and Validation Process



Proteins on Custom Assay Panel

Biomarkers in Custom Assay Panel and intersection with radiographic features



Biomarker	Name (Alias)
APLP1	Amyloid Beta Precursor Like Protein 1
CCL20	MIP-3 alpha
CD6	Cluster of Differentiation 6
CDCP1	CUB domain-containing protein 1
CNTN2	Contactin 2
COL4A1	Collagen alpha-1(IV) chain
CXCL13	C-X-C Motif Chemokine Ligand 13, BLC
CXCL9	Monokine Induced by Gamma Interferon, MIG
FLRT2	Leucine-rich repeat transmembrane protein
GFAP	Glial Fibrillary Acidic Protein
GH	Growth Hormone, Somatotropin
IL-12B	Interleukin 12B
MOG	Myelin-oligodendrocyte glycoprotein
NEFL	Neurofilament Light
OPG	Osteoprotegerin, TNFRSF11B
OPN	Osteopontin
PRTG	Protogenin
SERPINA9	Serpin Family A Member 9
TNFRSF10A	TRAILR1, DR5 - Death Receptor 5
TNFSF13B	BAFF
VCAN	Versican, versican proteoglycan

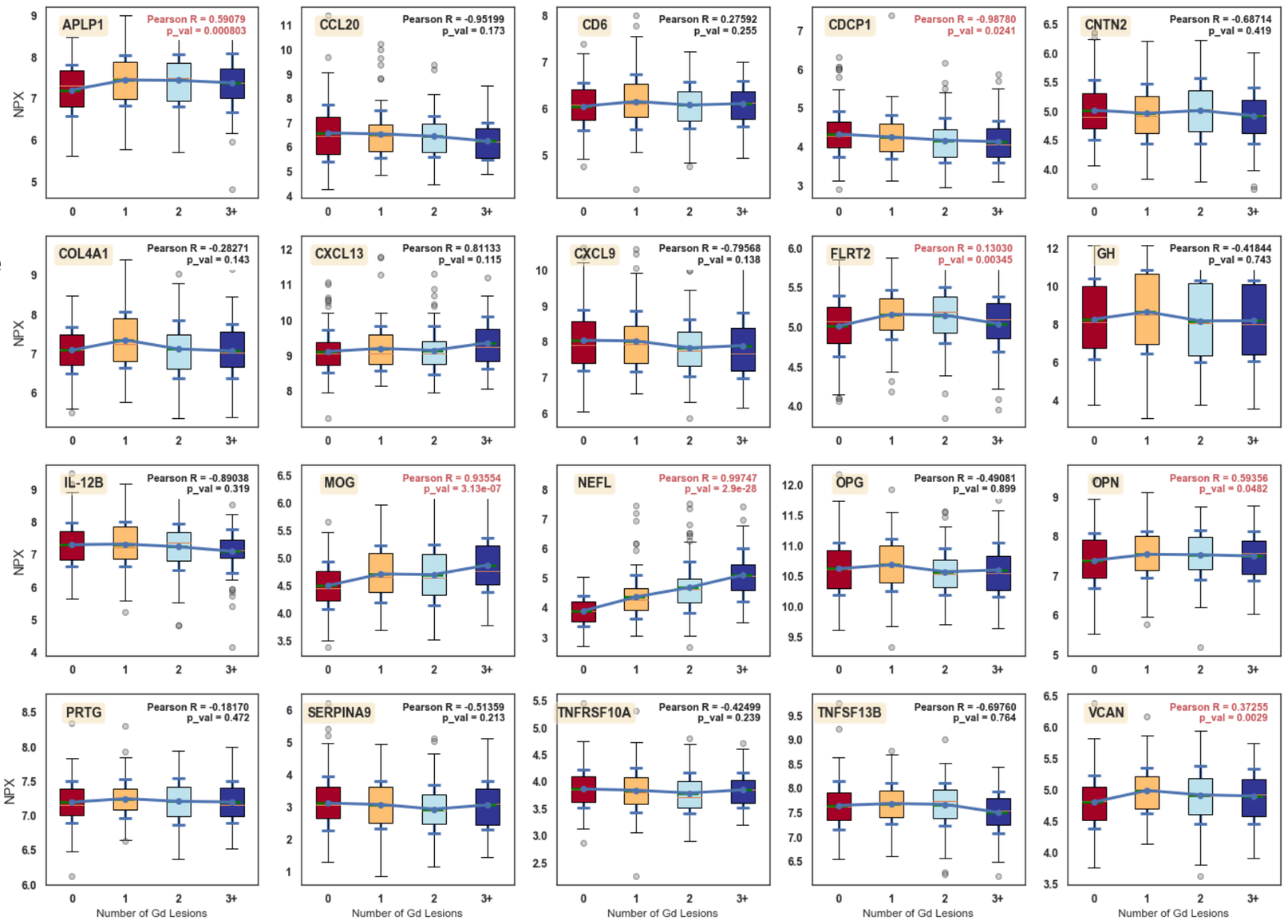
Pathway Analysis Procedure* (a) Spatial Expression via Human Protein Atlas and Allen Brain Atlas (b) Protein Functional Interactions via STRING and Cytoscape (c) Gene Set Enrichment via Enrichr

***Additional details in P0082: From Proteome to Interactome: A Mechanistic Approach to MS Biomarker Discovery**

Univariate Gd Lesion Count Analysis

- Data processing:
  - Discard samples taken more than 30 days apart from MRI scans.
  - Bridge normalization applied to account for relative quantitation batch-to-batch variability.
  - Raw NPX (normalized protein expression) levels shown. Features are demographically adjusted with respect to *age*, *sex,* and *disease duration* for multivariate modeling.
- 25% and 75% quantile for each NPX biomarker for 0 Gad lesions (red box), 1 Gad lesion (yellow box), 2 Gad lesions (cyan box), and 3+ Gad lesions (blue box).
- Pearson R value results from linear fitting the mean values (blue dots) with the increase of Gad lesions (clipped at 5)
- p-value results from 2-sample t-test comparing 0 vs. 1+ Gd lesion samples. Statistically significant features (p<0.05) have been highlighted in red.

Blended CLIMB + EPIC cohorts (n = 501)

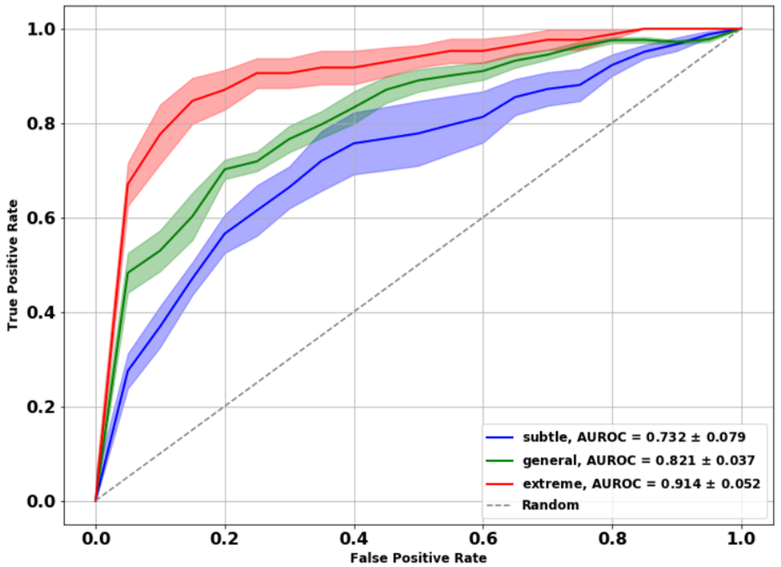


Gd+ Lesion - Univariate vs. Multivariate Analysis

Blended CLIMB + EPIC cohorts (n=468)

Gd Count Disease Activity Definitions: *Subtle* (0 vs 1), *General* (0 vs 1+), and *Extreme* (0 vs 3+)

Receiver Operating Characteristic Curve



Classification Procedure:

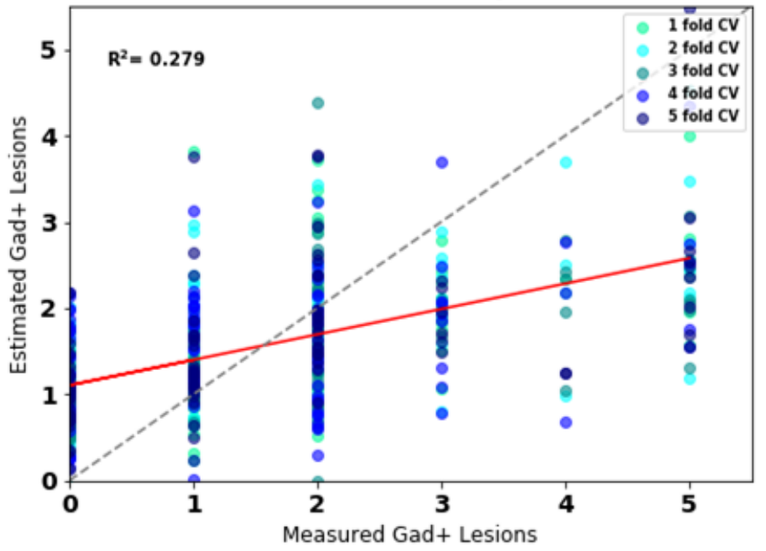
-OLS residuals Demographic-Adjustment

-Bridge normalization accounts for batch-to-batch variability

-Features in logistic regression model to classify Gd-based Disease Activity:

- NEFL
- IL-12B
- VCAN
- TNFRSF10A
- FLRT2
- CNTN2

Estimated vs. Measured Gad+ Lesions



Regression Procedure:

-Covariate-based Demographic-Adjustment

-Bridge normalization accounts for batch-to-batch variability

-Gd count imputed at 5

-Features in ridge regression model to predict Gd count:

- NEFL
- IL-12B
- CCL20
- TNFRSF10A
- TNFSF13B
- Age
- Disease Duration

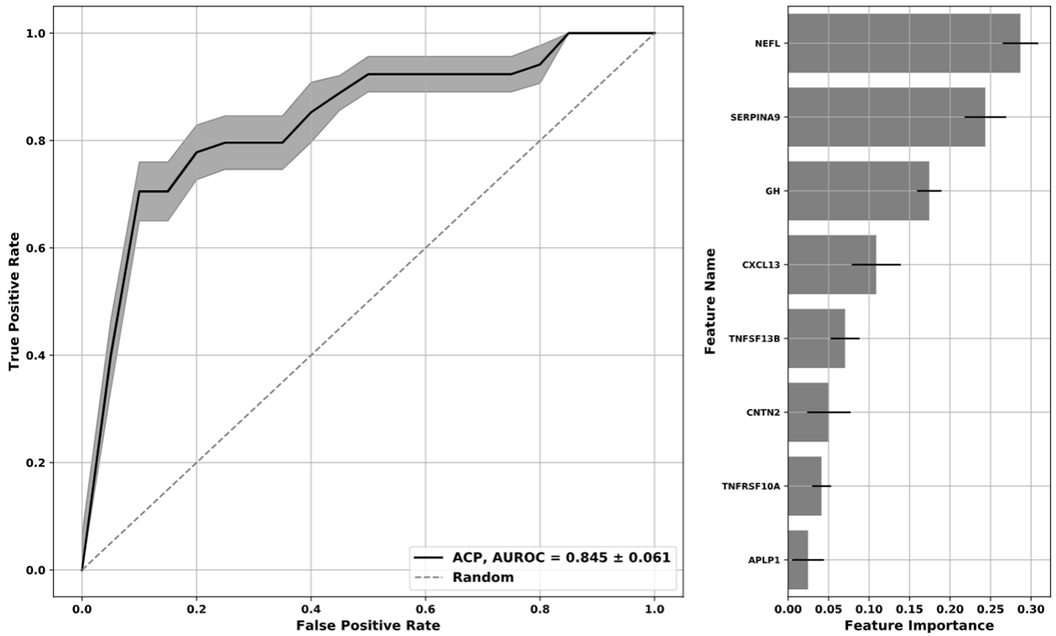
Gd+ Classification Comparison

	Subtle (AUROC)	General (AUROC)	Extreme (AUROC)	Regression (R^2)
Univariate NFL	0.697 ± 0.085	0.791 ± 0.046	0.890 ± 0.037	0.251 ± 0.020
Multivariate Model (best features)	0.732 ± 0.079	0.821 ± 0.037	0.914 ± 0.052	0.279 ± 0.022
Multivariate Model (without NFL)	0.701 ± 0.055	0.645 ± 0.075	0.734 ± 0.130	0.071 ± 0.018

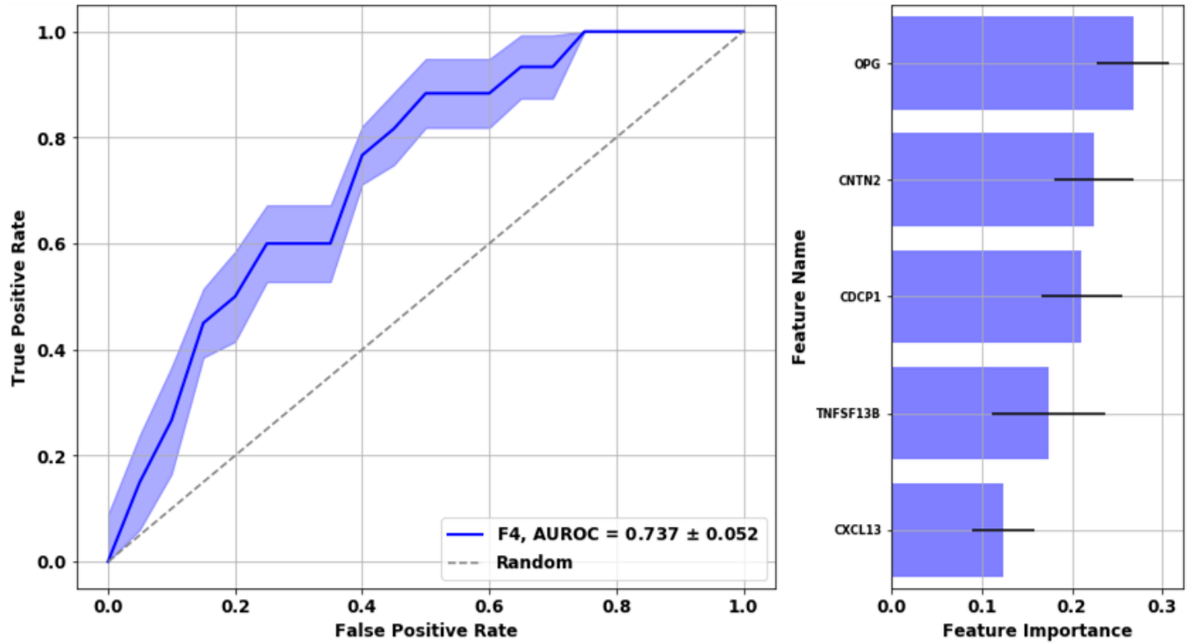
Model Performance estimated by splitting the dataset into training and test and applying 5-fold cross-validation.

Clinical Relapse Status & ARR

- ACP (n=124) samples from patients in a state of exacerbation (60) vs. quiescence (64)
- Significant univariate features (p-value): NEFL (0.00003), GH (0.002), SERPINA9 (0.002), FLRT2 (0.003), CNTN2 (0.008)
- Selected features in two ways:
  - Forward selection across demographically-adjusted custom assay features, and pick highest performer.
  - Train L1 regularized model, keep surviving features.



- CLIMB subset (n=168) with 148 samples from patients experiencing low (≤0.2) ARR vs. 20 samples with high (≥1.0) ARR
- Significant univariate features (p-value): NEFL (0.0258) *study underpowered due to ARR class imbalance
- Selected features in two ways:
  - Forward selection across demographically-adjusted custom assay features, and pick highest performer.
  - Train L1 regularized model, keep surviving features.



Conclusions & Discussion

- Multivariate models restricted to the 21 selected proteins for the custom assay panel effectively classified several radiographic and clinical endpoints.
- The 21-plex custom assay panel has been manufactured and is currently being analytically validated to establish the following specifications and parameters: Accuracy, Precision, Sensitivity, Specificity, Reference Ranges, Stability (reagents and samples), Diurnal Variation, Drug interference and Assay Robustness.
- Analytical Validation will be followed by clinical validation studies to verify association with Disease Activity endpoints (primary - Gadolinium-enhancing lesions) in multiple 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, (5) Response to DMTs
  - Expansion of the tests clinical utility to be investigated with future studies to evaluate biomarker correlations with endpoints associated with Disease Progression, Therapy Selection and Differential Diagnosis.

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