Abstract 7684
Section 39

Absolute Quantification of 400 Plasma Proteins Using a Novel NGS-Based Immunoassay: Analytical Validation and Biomarker Discovery in Breast Cancer

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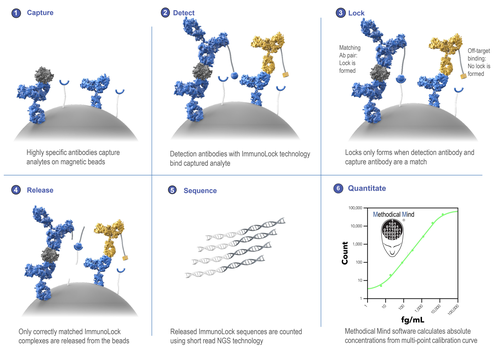
1 Introduction

Next generation sequencing (NGS)-based immunoassays have enabled high-multiplex, sensitive, wide dynamic range protein measurement, but existing platforms face a critical translational gap: proteomic discovery assays lack absolute quantitation and have no direct path to focused validation panels. We introduce a novel high multiplex immunoassay platform from ProteinXI, a division of Meso Scale Diagnostics, LLC. (MSD), enabling simultaneous absolute quantitation of hundreds of proteins with a direct translational route to MSD's high-throughput electrochemiluminescent (ECL) platform. Strong concordance with existing MSD U-PLEX and V-PLEX assays is demonstrated in plasma samples. Applied to a longitudinal neoadjuvant breast cancer cohort (N=111, 3 timepoints, 4 molecular subtypes) supplied by collaborators, the platform recapitulates known pharmacodynamic effects and identifies outcome-associated biomarkers.

2 ProteinXI Workflow

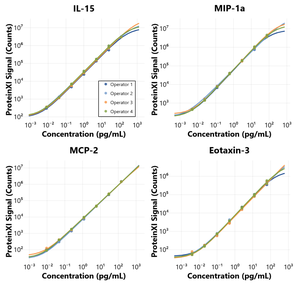
ProteinXI assays follow a simple 6-step process to quantify hundreds of proteins in 10 µL biofluid sample



3 Platform Evaluation – Sensitivity, Dynamic Range, and Precision

Each ProteinXI assay uses an 8-point calibration curve to enable absolute quantitation over a wide dynamic range. Analytical performance of 389 assays was assessed in a multi-operator study. Median limit of detection (LOD) was 1.29 pg/mL with 40 most sensitive assays achieving LOD ≤20 fg/mL (see table). 305 assays exceeded 4 Log(10) dynamic range (Median=4.96 Log(10)). Median intraplate CV and inter-operator CV were 6.55% and 8.63%, respectively.

Representative calibration curves demonstrate high sensitivity, dynamic range, and reproducibility



Most sensitive assays (n=40, LOD ≤20 fg/mL)

Assay	Avg LOD (fg/mL)	Max LOD (fg/mL)	Avg Dynamic Range (Logs)	Avg Hill Slope	Avg R^2 of 4PL fit
IL-15	1.3	2.2	5.8	1.01	1.000
MIP-1a	1.4	1.9	5.5	1.02	1.000
MCP-2	2.2	3.3	6.9	1.04	0.993
Eotaxin-3	3.1	5.8	6.0	1.02	0.998
MIP-1b	3.2	7.9	6.7	1.04	0.999
IL-3	4.3	5.8	5.6	1.03	0.999
GM-CSF	4.4	8.8	5.5	1.02	0.999
IL-2Ra	5.1	6.3	5.6	1.03	1.000
Eotaxin	5.4	17.1	6.7	1.03	0.999
IL-6	5.6	9.9	5.7	1	1.000
C1q receptor	6.1	10.3	6.7	1.02	1.000
TNF-b	6.2	6.8	5.4	1.03	1.000
IL-9	6.4	11.1	5.3	1.02	0.999
I-TAC	6.5	7.6	6.5	1.03	0.999
Fas soluble	6.6	11.7	5.4	1.02	1.000
IL-16	7.4	10	6.0	1.02	1.000
TNF-RI	7.4	13	5.1	1.03	1.000
PP	7.6	10.2	5.8	1.02	1.000
IL-10	7.9	13.6	5.0	1.02	1.000
TRAIL-1	8	11.1	5.4	1.03	1.000
IFN-b	8.1	12.5	5.6	1.02	0.999
IP-10	8.7	14	6.0	1.01	1.000
4-1BB1	9.1	10.8	5.8	1.02	1.000
CD40L soluble	9.1	14.8	6.7	1.02	1.000
G-CSF	9.1	19.8	4.6	1.04	1.000
Granzyme B	9.3	14	6.1	1.03	0.999
LYVE-1	11.7	21.6	6.7	1.03	1.000
HGF	11.8	28	5.7	1.03	0.999
RANTES	12.1	26.1	6.7	1.08	0.973
CD27	12.9	18.2	6.4	1.04	1.000
M-CSF	14.3	17.9	5.1	1.02	1.000
IL-12p70	15.4	25.8	5.1	1.02	1.000
BCA-1-BLC	15.5	42.5	7.0	1.06	0.994
CD21-CR2	16.2	32	5.4	1.03	1.000
IL-17D	16.5	19.8	5.1	1.04	1.000
IFN-g	17.5	23.1	5.9	1.03	1.000
IL-17B	17.5	31.3	5.5	1.03	0.999
CD276-B7-H3	19.1	34.1	5.1	1.02	1.000
GDF-15	19.8	63.3	5.2	1.04	1.000
IL-31	19.9	28.6	5.3	1.02	1.000

Low Intra-plate CV shows high assay precision

Intraplate CV by Panel

Panel	Space	N	Q1	Median	Q3	IQR
All	Concentration	42492	2.92	6.55	12.43	9.5
2X	Concentration	15856	2.86	6.43	12.35	9.49
40X	Concentration	11780	3.17	6.83	12.58	9.41
800X	Concentration	9831	2.83	6.4	12.46	9.63
16000X	Concentration	5025	2.82	6.41	12.19	9.37
All	Signal	47533	2.82	6.25	11.81	8.99
2X	Signal	20840	2.83	6.32	12.01	9.18
40X	Signal	11826	2.96	6.38	11.71	8.75
800X	Signal	9840	2.69	5.97	11.56	8.87
16000X	Signal	5027	2.72	6.17	11.82	9.09

Low Inter-operator CV shows excellent reproducibility

Inter-Operator CV by Panel

Panel	Space	N	Q1	Median	Q3	IQR
All	Concentration	9866	6.03	8.63	12.25	6.22
2X	Concentration	3661	7.44	10.31	13.75	6.3
40X	Concentration	2569	5.75	8.21	12.07	6.32
800X	Concentration	2384	4.79	6.92	9.97	5.19
16000X	Concentration	1252	5.96	8.24	11.11	5.15
All	Signal	10986	9.08	12.51	16.5	7.42
2X	Signal	4760	7.43	10.4	15.31	7.88
40X	Signal	2584	8.87	11.7	14.59	5.72
800X	Signal	2388	12.71	15.36	17.9	5.19
16000X	Signal	1254	11.81	14.4	16.95	5.14

4 Assay Paneling for Plasma

Assays were grouped into 4 dilution panels (2X, 40X, 800X and 16000X) based on typical analyte concentrations in plasma to maximize detectability and dilution linearity across the full dynamic range of plasma proteins. Performance was assessed in 37 commercially sourced healthy individual plasma samples.

93% detectable, 83.8% quantifiable in plasma

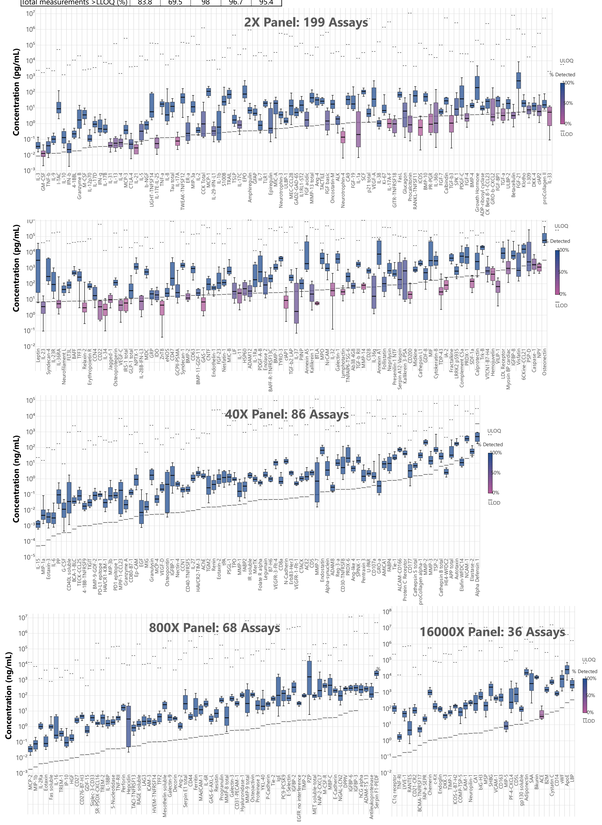
Detectability

	All	2X	40X	800X	16000X
Assays tested	389	199	86	68	36
Assays with >90% Detectability	317	130	86	66	35
Assays with >90% Quantifiable	271	95	79	63	34
Total measurements >LOD (%)	93.2	86.7	99.9	98.4	99.2
Total measurements >LLOQ (%)	83.8	69.5	98	96.7	95.4

Most assays dilute linearly in plasma

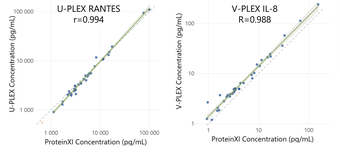
Dilution Linearity in Plasma

	All	2X	40X	800X	16000X
Assays tested	389	199	86	68	36
Assays w/80-125% Dilution Lin. (#)	317	138	84	67	33
Assays w/80-125% Dilution Lin. (%)	79.6	69.3	97.7	98.5	91.7



5 Assay concordance with U-PLEX and V-PLEX

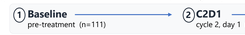
To validate cross-platform consistency, 37 healthy control plasma samples were measured on 118 U-PLEX assays and 27 V-PLEX assays overlapping with the ProteinXI Human Discovery 400 panel. Pearson correlations were calculated for all assays that were quantifiable on both platforms in at least 5 samples (see table). Median r exceeded 0.96 across both platforms, confirming strong concordance and a direct translational route from ProteinXI discovery to focused ECL validation panels. Assay sensitivity was also comparable between platforms, supporting the use of ECL platforms as a path to focused validation panels.



Platform	Assays Tested	Assays Analyzable (n ≥5)	Pearson Correlation Coefficient (r) 25th Percentile	Median	75th Percentile
U-PLEX	118	88	0.86	0.95	0.98
V-PLEX	27	19	0.92	0.97	0.99

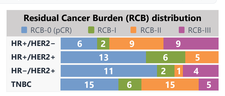
6 Translational Relevance: Neoadjuvant Breast Cancer Study Design

Plasma was collected from 111 patients with invasive breast cancer receiving neoadjuvant chemotherapy and subtype-appropriate targeted therapy at two sites: Washington University in St. Louis (n=84) and University of Miami (n=27). Three longitudinal timepoints were collected: Baseline (pre-treatment), C2D1 (Cycle 2, Day 1; early on-treatment), and Pre-Surgery (post-treatment). Molecular subtypes were defined by immunohistochemistry: HR+ (ER+ or PR+), HR- (ER- and PR-), HER2+, HER2-, and derived groups (HR+/HER2-, HR+/HER2+, HR-/HER2+, Triple Negative Breast Cancer (TBNC). Treatment response was assessed by pathologic complete response (pCR) and Residual Cancer Burden (RCB) group. De-identified samples were provided to MSD.



Subtype	N	WashU	UM	pCR rate
HR+/HER2−	26	20	6	23%
HR+/HER2+	24	19	5	54%
HR−/HER2+	18	11	7	61%
TNBC	41	32	9	37%
Total	**111***	**84**	**27**	**41%**

Residual Cancer Burden (RCB) distribution



7 Platform Performance in Cancer Plasma

Samples were analyzed with the ProteinXI Human Discovery 400 panel as described in sections 2-4. Calibrators were run in duplicate on each plate; controls and samples were tested in single replicates. Control recovery and precision met pre-specified acceptance criteria. Detectability in cancer plasma was comparable to healthy controls (83.2% vs. 83.8% quantifiable). Assays with fewer than 20% of samples above LLOQ were excluded from statistical analyses (350 assays retained).

Calibrator and Control Performance Metrics

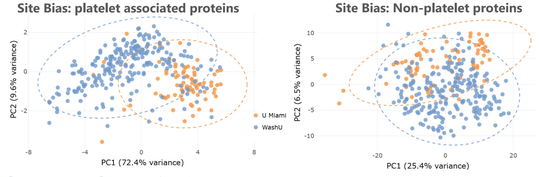
	Q1	Median	Q3	IQR
Calibrator Intraplate CV (%)	2.9	6.8	14.8	11.9
Calibrator Interplate CV (%)	2.9	4.7	9.2	6.4
Control Interplate CV (%)	7.4	12.5	18.6	11.2
Control Recovery (%)	78.4	89.9	101.1	22.7

Detectability

	All	2X	40X	800X	16000X
Assays tested	392	202	86	68	36
Assays with >90% Detectability	305	130	86	66	35
Assays with >90% Quantifiable	265	95	79	63	34
Total measurements detectable (%)	93.10%	86.3	99.9	98.4	99.2
Total measurements quantifiable (%)	83.20%	68.9	98	96.7	95.4

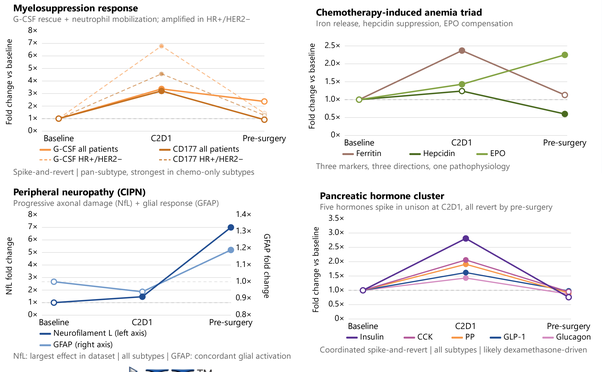
8 Pre-Analytical Assessment

Principal component analysis revealed a site-specific batch effect: platelet degranulation markers were elevated in all three timepoints of the University of Miami samples, consistent with documented sample processing delays at that site (left panel). Significant site-bias persisted across the broader proteome after exclusion of platelet-associated proteins (right panel). As pre-analytical and biological variation could not be sufficiently distinguished, samples collected at University of Miami (n=27) were excluded from further analyses; samples collected at Washington University (n=84) were retained.



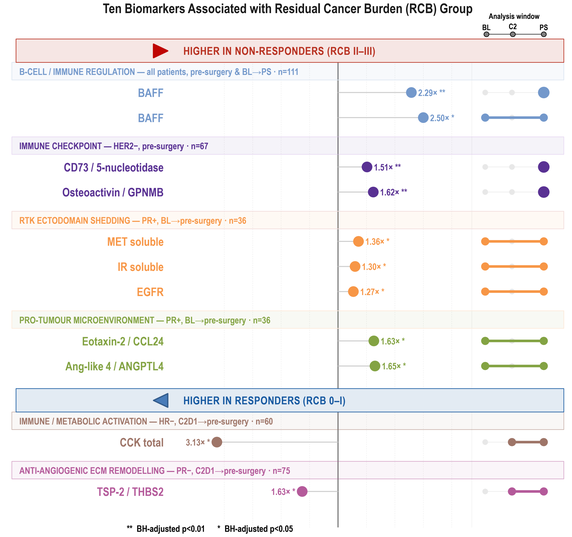
9 Pharmacodynamic Reponses

ProteinXI assays capture expected pharmacological consequences of neoadjuvant chemotherapy through within-subject paired analysis (Wilcoxon signed-rank test with Benjamini-Hochberg (B-H) adjustment at 5% false discovery rate across 350 quantifiable assays per timepoint pair). Signals were reproducible across all molecular subtypes, with subtype-specific amplification observed in the myelosuppression response. Plotted values are median ratios relative to baseline and those with B-H adjusted p-values <0.01 are shown as solid dots with non-significant points as open dots.



10 Biomarkers Associated with Treatment Response

Response-associated biomarkers were identified through a whole-panel batch analysis (350 assays x 11 molecular subtype grouping x 3 outcome groupings x 3 single-timepoint and 3 longitudinal change analyses), with B-H adjustment applied per analysis pass (FDR <0.05). Single-timepoint analyses compared concentration distributions between outcome groups at each timepoint. Change analyses computed within-individual paired fold change (FC) for individuals with observations at both timepoints, then tested FC distributions between outcome groups. Two primary outcome groupings were considered: Residual Cancer Burden (RCB) 0/I vs RCB II/III, which classifies individuals as strong responders (RCB 0 or RCB I) versus poor responders (RCB II or RCB III) and pathologic complete response (pCR), defined as Complete (RCB 0) vs Incomplete (RCB I, RCB II, or RCB III). In cases where a biomarker was only significant for one of the two primary outcome groupings we also considered a secondary grouping of RCB 0 vs. RCB II/III as a confirmatory test. Ten markers that were significant in at least one primary plus the confirmatory grouping are reported here.



11 Summary: Sample QC to Response Biomarkers in a Single Assay Run

ProteinXI — Biomarker 400 Panel

Absolute quantitation of 400 plasma proteins from 10 µL EDTA plasma sample · 111 patients · 3 timepoints

Sample QC
Each timepoints

Identified likely sources of pre-analytical variability:
- Platelet activation
- Likely hemolysis

Identified other possible bias between collection sites:
- Population differences: adiposity, diet, metabolic
- Treatment differences

Pharmacodynamics
BL → C2; C2 → Pre-Surgery

Recapitulated 4 well-known effects of chemotherapy:
- Myelosuppression
- Chemo-induced anemia
- Peripheral neuropathy (CIPN)
- Disruption of metabolic hormones

Outcome: Single timepoint
Pre-Surgery Timepoint

Identified biomarkers that were elevated at pre-surgery timepoint in non-responding populations
- BAFF elevation may indicate non-productive humoral response; observed across subtypes
- CD73 and Osteoactivin/GPNMB may act as immune checkpoints in HER2- population

Outcome: Change
BL → Pre-Surgery; C2 → Pre-Surgery

Identified biomarkers that increased throughout treatment in non-responding populations
- Receptor tyrosine kinases increase in receptor+ non-responders
- Eotaxin 2 and ANGPTL4 indicate pro-tumor microenvironment

Biomarkers that increased late in treatment in responders
- CCK increase and TSP-2 increase may indicate immune activation and anti-angiogenic remodeling

12 Conclusion

The ProteinXI Human Discovery 400 panel enables simultaneous absolute quantitation of hundreds of plasma proteins from a single 10 µL aliquot, with analytical performance comparable across healthy and cancer plasma samples. Applied to a longitudinal neoadjuvant breast cancer cohort, the platform captured expected pharmacodynamic consequences of chemotherapy across multiple organ systems, confirming sensitivity to known biological signals, and enabled identification of ten outcome-associated biomarkers. These results demonstrate the ProteinXI platform's robust analytical performance and ability to reproduce expected biological patterns, supporting its translational utility for biomarker discovery in oncology.

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