Development and Validation of a Multi-Analyte Profile Test for Broad Applications in Oncology:

Funded by The National Cancer Institute and to be expanded by CPRIT

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Introduction to RBM

Rules-Based Medicine® (RBM) is a CLIA-certified biomarker testing laboratory that uses multiplexed immunoassays to provide innovative products and services. Enabled by microsphere technology and decades of experience, our proprietary Multi-Analyte Profiling (MAP) platform makes drug discovery and development more efficient and effective. Using extremely small sample volumes, we provide discovery, pre-clinical and clinical researchers with reproducible, quantitative, multiplexed immunoassay data for hundreds of biomarkers. The RBM approach utilizes DiscoveryMAP®, our largest MAP providing quantitative data for 266 biomarkers, as a hypothesis-generating tool in the primary study. Once identified, the biomarker pattern of interest is evaluated in subsequent studies to validate its utility. Data acquired help support subsequent trial designs and critical go/no go decisions. RBM's fully automated platform is analytically validated to clinical laboratory standards that we provide as a service to pharmaceutical, biotech and academic investigators. The RBM approach to biomarker testing delivers advantages that are changing the way therapeutics are developed, people are diagnosed and patients are treated.

Introduction to OncologyMAP™

The OncologyMAP service delivers data from the quantitative measurement of 102 cancer-related serum proteins as defined by Polanski and Anderson in a 2006 review (Biomarker Insights 2006:1 1-48). Development and validation of the majority of these assays was funded by the Small Business Innovation Research program of the National Cancer Institute.* This novel service offering is an unprecedented tool for broad applications in oncology.

Aims of our CPRIT Program

CPRIT has awarded RBM $3.1M to develop and validate an additional 150-180 multiplexed immunoassays for cancer-related biomarkers over the next 36 months. The review article referenced above will be the blue print for the assay development list. However, we are inviting all CPRIT investigators across Texas to contribute their suggestions for the construction of the list.

Assay validation

RBM's validation parameters include sensitivity, dynamic range, linearity, cross reactivity, matrix effects and precision. Please see
An Overview of Assay Quality Systems at Rules-Based Medicine®, QA/QC White Paper By Brian T. Welsh, Ph.D. and James Mapes, Ph.D. for further information on RBM's validation procedures.

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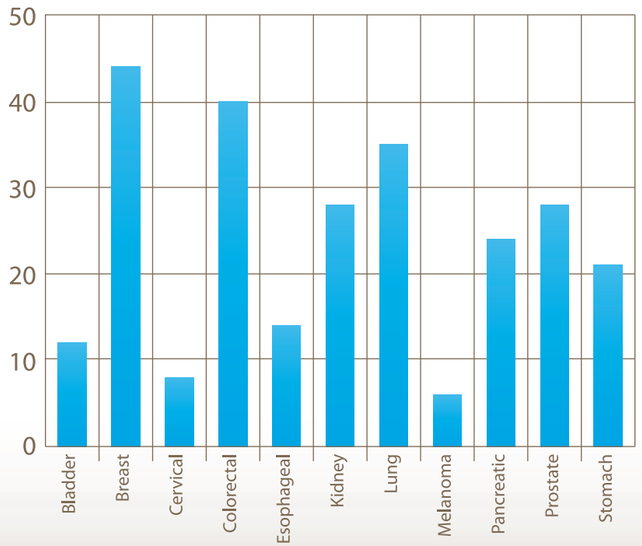
Differentiation of cancer serum samples using OncologyMAP™

Once developed and validated, the 102 assays of OncologyMAP version 1.0 were applied to 533 serum samples from normal matched controls and 11 broad groups of cancer patients. The vast majority of these cancer samples were collected from patients in stage II/III of their disease.
This was meant to demonstrate the utility of such testing and was not meant as clinical validation. Much work is needed now that the test is available.

Numbers of each group of cancer samples tested with OncologyMAP™

Bladder	17	**Lung**	50
Breast	49	**Melanoma**	11
Cervical	22	**Pancreatic**	12
Colorectal	51	**Prostate**	94
Esophageal	5	**Stomach**	24
Kidney	24	**Controls**	174

Numbers of significantly different analytes measured in these cancer samples versus controls (p<0.001)



Sensitivity and Imprecision of the 102 assays in OncologyMAP™

z	Analyte	Units	LDD	CVs
1	6Ckine	pg/mL	5.8	5%
2	Aldose Reductase	ng/mL	2.64	6%
3	Alpha-Fetoprotein	ng/mL	0.328	3%
4	Amphiregulin	pg/mL	320	3%
5	Angiogenin	ng/mL	1.95	5%
6	Annexin A1	ng/mL	450	7%
7	B cell-activating factor	pg/mL	20	7%
8	B Lymphocyte Chemoattractant	pg/mL	33	6%
9	Bcl-2-like protein 2	ng/mL	2.3	9%
10	Betacellulin	pg/mL	323	8%
11	Calprotectin	ng/mL	29.1	7%
12	Cancer Antigen 125	U/mL	1.87	4%
13	Cancer Antigen 15-3	U/mL	0.439	4%
14	Cancer Antigen 19-9	U/mL	0.492	4%
15	Cancer Antigen 72-4	U/mL	4.04	4%
16	Carcinoembryonic Antigen	ng/mL	0.12	3%
17	Cathepsin D	ng/mL	34.7	6%
18	Cellular Fibronectin	ug/mL	0.353	9%
19	Collagen IV	ng/mL	7.27	7%
20	Endoglin	ng/mL	0.017	8%
21	Endostatin	ng/mL	0.42	4%
22	Eotaxin-2	pg/mL	33.3	4%
23	Epidermal Growth Factor	pg/mL	8.3	9%
24	Epidermal Growth Factor-R	ng/mL	0.045	5%
25	Epiregulin	pg/mL	30	6%
26	Epithelial cell adhesion molecule	pg/mL	65	4%
27	Ezrin	ng/mL	19	7%
28	FABP, adipocyte	ng/mL	0.11	3%
29	FABP, liver	ng/mL	8.2	4%
30	Fibroblast Growth Factor basic	pg/mL	12.6	5%
31	Fibulin-1C	ug/mL	0.06	5%
32	Galectin-3	ng/mL	2.5	7%
33	Gelsolin	ug/mL	0.444	5%
34	Glucose-6-phosphate Isomerase	ng/mL	57.8	16%
35	GCLR	ng/mL	10.9	12%
36	Glutathione S-Transferase Mu 1	ng/mL	12.2	6%
37	HE4	pM	81.8	2%
38	HB-EGF	pg/mL	22.5	5%
39	Hepatocyte Growth Factor	ng/mL	0.354	4%
40	Hepatocyte Growth Factor-R	ng/mL	0.199	4%
41	Hepsin	pg/mL	58.5	5%
42	hCG	mIU/mL	2.69	4%
43	HER-2	ng/mL	0.001	4%
44	IGFBP-1	ng/mL	0.456	6%
45	IGFBP-2	ng/mL	5.8	4%
46	IGFBP-3	ng/mL	35	8%
47	IGFBP-4	ng/mL	4.3	4%
48	IGFBP-5	ng/mL	9.2	7%
49	IGFBP-6	ng/mL	14	8%
50	IP-10	pg/mL	52.8	5%
51	ITAC	pg/mL	33.7	5%

z	Analyte	Units	LDD	CVs
52	Interleukin-2 -R alpha	pg/mL	307	6%
53	Interleukin-6	pg/mL	1.36	4%
54	Interleukin-6 -R subunit beta	ng/mL	10.15	6%
55	Kallikrein 5	ng/mL	0.151	8%
56	Kallikrein-7	pg/mL	449	6%
57	Lactoylglutathione lyase	ng/mL	0.15	5%
58	LAP TGF-b1	ng/mL	0.056	4%
59	Leptin	ng/mL	0.145	3%
60	MIP-3 beta	pg/mL	37.2	4%
61	MIF	ng/mL	0.034	4%
62	Macrophage-Stimulating Protein	ng/mL	14.8	3%
63	Maspin	pg/mL	2330	3%
64	Matrix Metalloproteinase-2	ng/mL	16.3	5%
65	Mesothelin	nM	1.59	3%
66	MICA	pg/mL	50	4%
67	Monocyte Chemotactic Protein 1	pg/mL	36.3	5%
68	MIG	pg/mL	36.4	7%
69	Neuron Specific Enolase	ng/mL	11	4%
70	Neuropilin-1	ng/mL	12.02	2%
71	NGAL	ng/mL	46.9	3%
72	Nucleoside diphosphate kinase B	ng/mL	1.33	8%
73	Osteopontin	ng/mL	0.751	4%
74	Osteoprotegerin	pM	0.552	6%
75	Pepsinogen I	ng/mL	34.7	8%
76	Peroxiredoxin 4	ng/mL	0.068	5%
77	Phosphoserine Aminotransferase	ng/mL	0.822	4%
78	Placenta Growth Factor	pg/mL	17.6	7%
79	Platelet-Derived Growth Factor BB	pg/mL	149	5%
80	Prostasin	ng/mL	1.42	6%
81	Protein S100-A4	ng/mL	6.29	15%
82	Protein S100-A6	ng/mL	7.41	9%
83	ErbB3	ng/mL	0.030	3%
84	SCCA-1	ng/mL	0.244	6%
85	Stromal cell-derived factor-1	pg/mL	52.6	4%
86	Tenascin-C	ng/mL	15	4%
87	Tetranectin	ug/mL	0.807	13%
88	Thyroglobulin	ng/mL	1.5	4%
89	Tissue type Plasminogen activator	ng/mL	0.12	4%
90	TGF-alpha	pg/mL	10.2	6%
91	Tumor Necrosis Factor -R I	pg/mL	52.3	4%
92	TIE-2	ng/mL	0.070	4%
93	uPA	pg/mL	69	5%
94	uPAR	ng/mL	0.12	4%
95	VEGF	pg/mL	20	4%
96	VEGF-B	ng/mL	13.7	6%
97	VEGF-C	ng/mL	0.46	6%
98	VEGF-D	pg/mL	500	4%
99	VEGFR-1	pg/mL	17	12%
100	VEGFR-2	ng/mL	0.041	5%
101	VEGFR-3	ng/mL	1.0	4%
102	YKL-40	ng/mL	4.0	3%

CPRIT Funding

Using funding from CPRIT, we are expanding the OncologyMAP to ~350 cancer-related proteins, thereby empowering the global research community to accelerate the pace of discovery, validation and translation of cancer biomarkers into clinically useful tests.