Multiplex bead array approach for quantitative profiling of 95 cancer-immunity biomarkers in breast cancer

Wen-Rong Lie[1], Alexander Davies[2], Danielle Pepin[1], Michael Godeny[1]. [1]**MilliporeSigma**, St. Louis, MO; [2]**The Ohio State University**, Columbus, OH

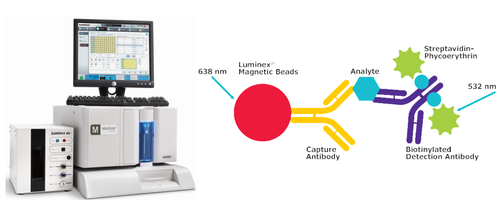
Abstract

Immune regulators, including immune checkpoint proteins and cytokines/chemokines, have an emerging role as both disease/treatment biomarkers and targeted agents in cancer therapies. To contribute to the understanding of the roles of these immune regulators in cancer, we have developed 3 new Luminex®-based multiplex immunoassay panels, a 48-plex human cytokine/chemokine panel, a 17-plex human immune checkpoint protein panel, and a 31-plex human immune checkpoint protein panel, to simultaneously quantitate the expression levels of 95 key immune regulator proteins in biofluids or cell/tissue homogenates. Here we report the quantitative profiles of these 95 immune regulators in 3 types of breast cancer samples: cancer versus healthy control serum samples, lysates from breast cancer tumor biopsies versus adjacent normal tissues, and conditioned media from established cancer cell lines. Analysis of the circulating immune protein signatures generated from this multiplex approach reveals an elevated level of IL-6, IL-27, M-CSF, MDC, MIG, sCD27, sTIM-3, sCD40, Galectin-3, Galectin-1, FGL-1, BAFF and low levels of EGF and sCD40L in breast cancer serum samples compared to the healthy serum controls. The expression profiling of tumor and adjacent normal tissues from 3 patients with metastatic breast cancer revealed differential expression of multiple protein markers including IL-6, IL-8, MIG, IL-1RA, IL-18, IP-10, MCP-1, MIP-1β, VEGF-A, BTLA, HVEM, CTLA-4, CD40, TLR-2, Siglec-9, CD25, Granzyme B, APRIL, BAFF, Nectin-2, Nectin-4, E-cadherin, and IDO-1 in the matched lysates. We also performed a similar analysis using the conditioned media of cultured breast tumor cells. Altogether, our results suggest Luminex®-based profiling allows for sensitive and versatile multiplexed analysis of stimulating and inhibitory immune mediators in circulation, tissues, and cell lines which may assist in the discovery of biomarkers and therapeutic targets for cancer interventions.

Methods

Sera, Cells, and Tissue: Human serum samples were purchased from Discovery Life Sciences, Inc. and BioreclamationIVT (BioIVT), including 24 breast cancer serum samples, and 24 normal serum samples. All cell lines listed were obtained from ATCC. Human breast cancer tissue samples, and the corresponding adjacent normal breast or colorectal tissue samples were obtained from Asterand. Tissue lysates were prepared in MILLIPLEX® Cell Signaling Lysis Buffer (Cat. No. 43-040).

Multiplex Protein Biomarker Immunoassays: Immune checkpoint protein and cytokine/chemokine biomarker profiles were quantitatively determined using the MILLIPLEX® Human Immuno-Oncology Checkpoint Protein Panel 1 (Cat. No. HCKP1-11K), Human Immuno-Oncology Checkpoint Protein Panel 2 (Cat. No. HCKP2-11K), and Human Cytokine/Chemokine /Growth Factor Panel A (Cat. No. HCYTA-60K). Samples were analyzed on a Luminex® 200™ System with MILLIPLEX® Analyst 5.1 software.



Results

Figure 1. Human Immuno-Oncology Checkpoint Protein Panel 2 (31-plex). This figure shows the I/O-2 multiplex standard curves for the simultaneous quantification of these 31 analytes.

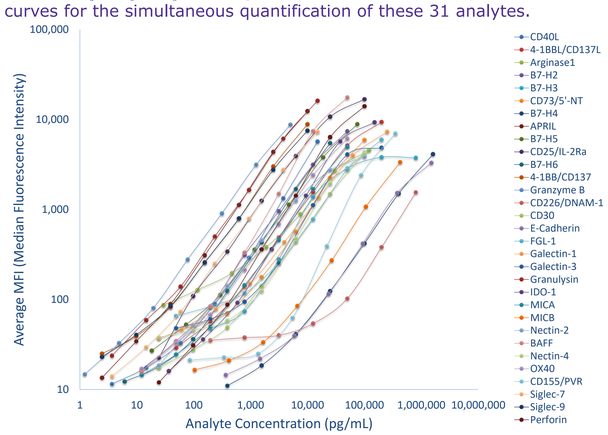


Figure 2. Human Immuno-Oncology Checkpoint Protein Panel 1 (17-plex). This figure shows the I/O-1 multiplex standard curves for the simultaneous quantification of these 17 analytes.

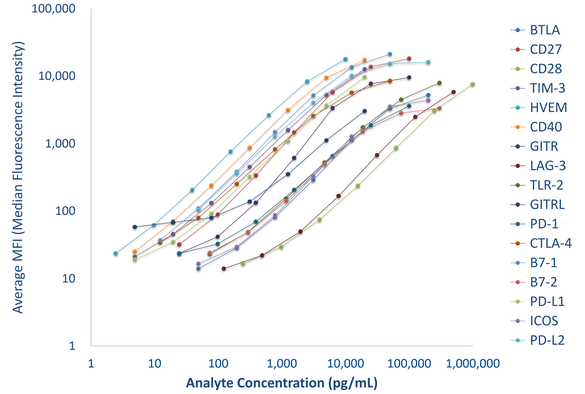


Figure 3. Human Cytokine/Chemokine/Growth Factor Panel A (48-plex). This figure shows the Panel A multiplex standard curves for the simultaneous quantification of these 48 analytes.

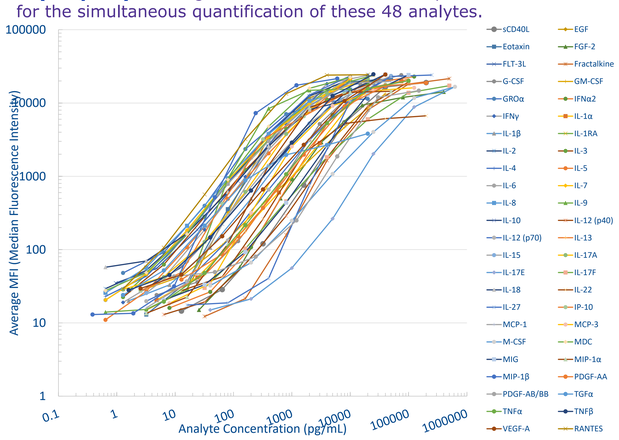


Figure 4. Multiplex assay utility in detecting circulating cancer-immunity biomarkers. Examples of the use of these immuno-oncology multiplex immunoassays for detecting soluble immune checkpoint proteins and cytokines/chemokines in sera from breast cancer patients (BrCa in red, n=24) and healthy control individuals (Cont in blue, n=24). The figure shows differential expression of 15 cancer-immunity biomarkers in these two groups of serum samples.

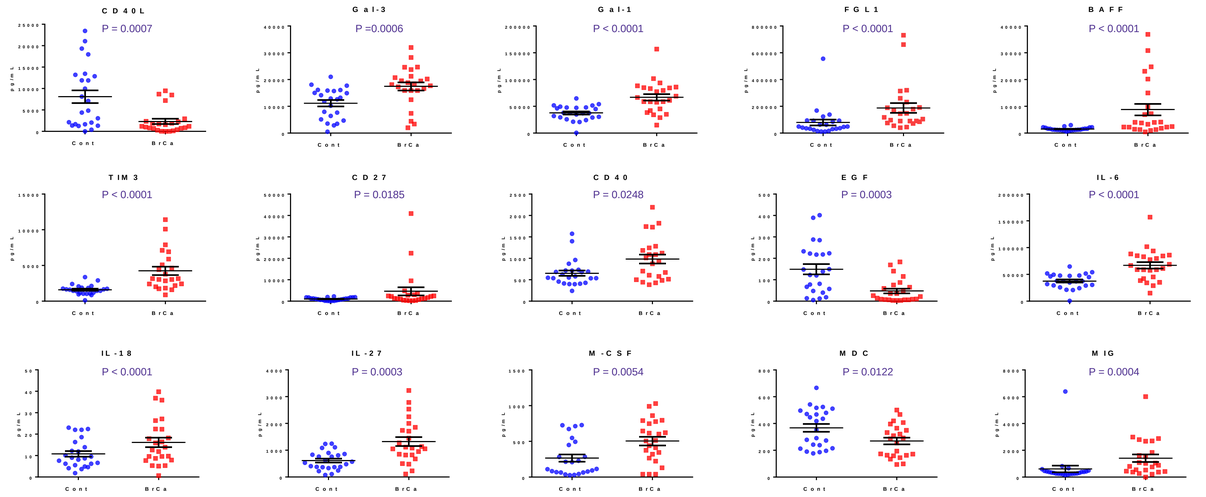


Figure 5. Multiplex assay utility in detecting cellular proteins in the lysates of tumor and adjacent normal tissues collected from patients. A heat map analysis of selective immune checkpoint proteins and cytokines/chemokines in lysates of tumor and adjacent normal tissues from patients with breast cancer (n=3). The data is presented as log2 of the concentration in a Red-Yellow-Blue heat map showing higher values in Red and lower values in Blue.

		IL-6	IL-8	MIG	IL-1ra	IL-18	IP-10	MIP-1b	VEGF	BTLA	HVEM	CTLA-4	CD40	TLR-2	APRIL	BAFF	Nectin-2	Nectin-4	E-Cad	IDO1
BrCa	Normal 1	-0.4	0.1	2.2	1.2	0.8	1.5	0.1	0.8	2.3	2.4	0.8	2.8	2.3	2.1	1.5	2.3	1.1	2.6	2.7
Pair 1	Tumor 1	0.4	1.4	3.1	2.7	1.8	3.1	1.1	1.8	3.2	2.7	1.7	3.1	3.0	3.2	2.2	2.8	2.4	3.7	2.4
BrCa	Normal 2	-0.5	0.5	3.1	0.0	0.6	2.1	0.3	1.5	1.5	2.1	1.0	2.9	2.7	2.3	1.6	2.0	1.6	2.5	2.5
Pair 2	Tumor 2	2.1	2.4	4.0	2.1	1.7	5.0	1.7	3.4	2.7	2.8	2.4	3.6	3.3	3.3	2.5	2.7	2.9	3.1	3.8
BrCa	Normal 3	-1.0	0.6	2.3	1.7	0.7	1.5	0.1	0.7	2.2	2.0	1.0	2.5	1.7	2.2	1.5	2.5	1.6	2.8	2.8
Pair 3	Tumor 3	0.7	1.5	3.6	2.5	2.3	2.5	1.2	2.2	1.7	3.1	2.0	3.5	3.0	2.9	2.3	3.2	2.7	4.1	2.9

Summary

We developed a new immuno-oncology checkpoint protein multiplex bead array (MILLIPLEX® Human Immuno-Oncology Checkpoint Protein Panel 2) for simultaneously quantifying 31 immune checkpoint proteins using 25µL of diluted samples for cancer and immunology research. MILLIPLEX® profiling of these 31 checkpoint proteins in combination with the 17 immune checkpoint proteins in Human Immuno-Oncology Checkpoint Protein Panel 1 and the 48 cytokines/chemokines in Human Cytokine/Chemokine/Growth Factor Panel A has identified secreted and cellular checkpoint protein and cancer-immunity biomarker candidates in breast cancer patient sera, tumor cell conditioned media (not shown), and human tumor tissue.

We reported 15 putative circulating cancer biomarkers in human breast cancer patient serum samples.

- Gal-3, Gal-1, FGL1, BAFF, sTIM-3, sCD27, sCD40, IL-6, IL-18, IL-27, M-CSF, MIG are elevated in human breast cancer patient sera when compared to the expression levels in sera from healthy controls.
- sCD40L, EGF, MDC are reduced in human breast cancer sera when compared to the expression levels in sera from healthy controls.

Differential expression of multiple checkpoint proteins were detected in breast cancer as cellular proteins in the lysates of tumor and adjacent normal tissues collected from patients.

Our results showed the combination of these three MILLIPLEX® Human Immuno-Oncology Checkpoint Protein Panel 1 (Cat. No. HCKP1-11K), Human Immuno-Oncology Checkpoint Protein 2 (Cat. No. HCKP2-11K), and Human Cytokine/Chemokine/Growth Factor Panel A (Cat. No. HCYTA-60K) is an ideal tool for studying immuno-oncology in cancer research. It allows simple, sensitive, reliable, and accurate biomarker identification/characterization and quantitative profiling of 95 biologically important changes.

www.sigmaaldrich.com/milliplex