APPLICATION NOTE

Uniquely Capturing the High Dynamic Range of Spatial Biomarkers With Imaging Mass Cytometry

Enabling predictive patient stratification, biomarkers of response and mechanism of action

Introduction

The success of therapeutic development relies on the thorough detailing of mechanisms of action and biological response. Measuring the levels of relevant proteins provides needed insight for these applications. With a greater dynamic range than fluorescence imaging technologies, Imaging Mass Cytometry™ (IMC™) enables the detection of distinct expression levels to identify critical proteins.

This application note outlines:

- Overcoming challenges in measuring protein expression levels
- Addressing limitations of fluorescence imaging with IMC
- Capturing the dynamic range of spatial biomarkers to enable predictive patient stratification
- Detecting biomarkers that indicate positive response to checkpoint combination therapy

Overcoming challenges in capturing high and low protein expression: Why dynamic range matters in spatial proteomics

Deciphering both therapeutic and immune responses is a difficult undertaking, but both are critical to accurately determine safety and efficacy of a potential therapy.

In this context, detection and monitoring of impacted proteins can be challenging depending on their abundance within the microenvironment.

Key to analytic success is the ability to measure the distinct expression levels of these proteins because the degree of underexpression or overexpression of a specific protein could uncover the difference in predicted response. For example, overexpression of certain antigens, such as HER2 in breast cancer, directly influences the aggressiveness of the tumor and helps guide treatment strategies.

While immunofluorescence methods are limited by low dynamic range leading to early saturation of signals, IMC is uniquely positioned for assessing high and low expression levels due to its high dynamic range.

IMC scales with the highest dynamic range, highest multiplex and highest throughput

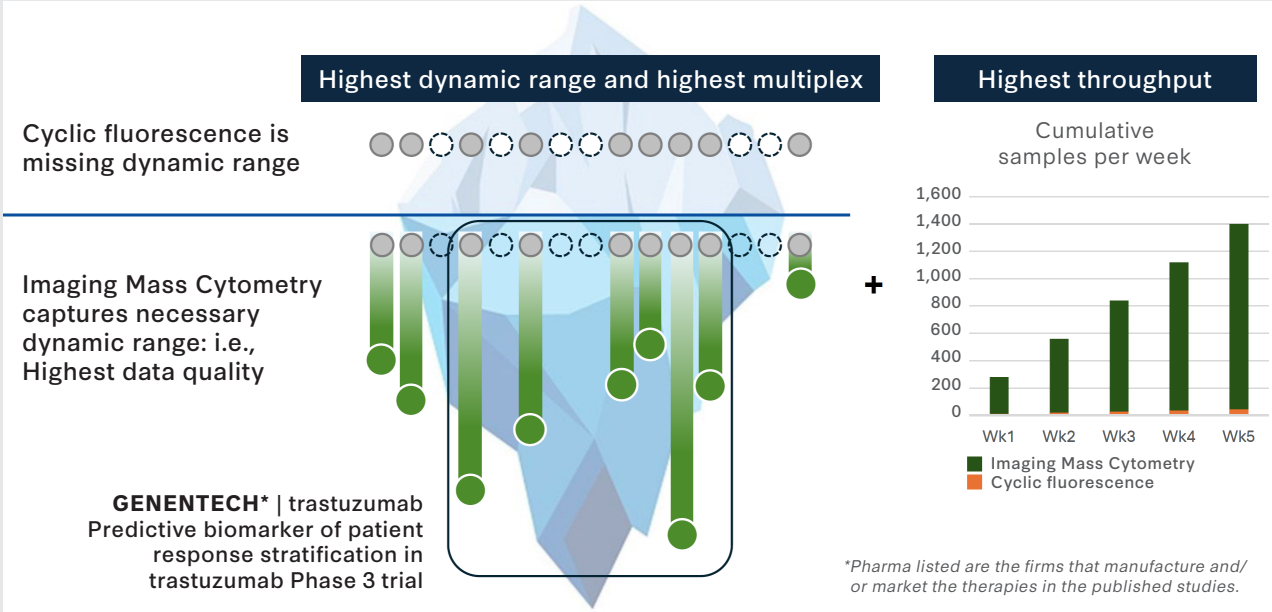

Figure 1. IMC enables unique patient stratification via its ability to capture the dynamic range of spatial proteomics. Cyclic fluorescence is unable to capture the dynamic range of spatial biomarkers. Additionally, IMC has up to 100x higher throughput than cyclic fluorescence.

"[quantitative immunofluorescence] has less dynamic range than IMC and appears to be saturated."[1]

IMC reveals the full range of protein expression, enabling immune insights unable to be captured with cyclic fluorescence

A high dynamic range better matches the already high biological dynamic range of the proteome, providing the capability to capture high- and low-expressing proteins. This enables the assessment of expression level without saturation of signals rather than relying on presence or absence, providing the opportunity to:

- Acquire accurate relative quantification of biomarkers without the need to compensate for autofluorescence
- Allow cell identification
- Characterize cells based on specific expression levels

For example, HER2 grading is used to determine HER2-directed therapies and is performed by assessing the level of HER2 expression in patient samples. The ability to distinguish between HER2 grades, with levels of 0, 1+, 2+ and 3+, is critical for determining whether a sample qualifies for these therapies.

IMC captures 50-plus markers simultaneously

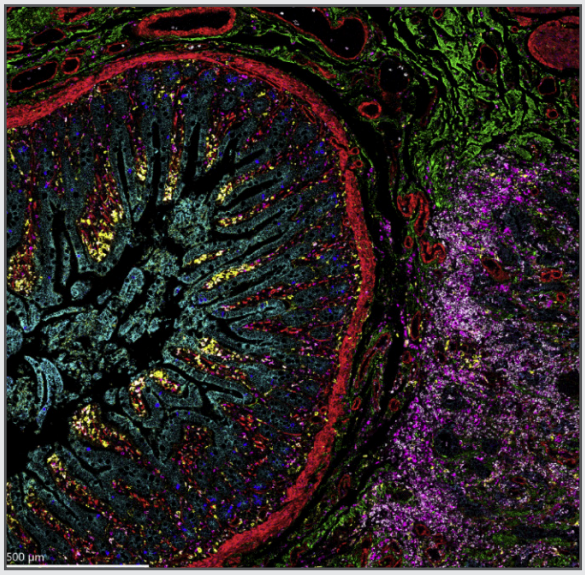

Figure 2: By capturing the full dynamic range of protein expression with 50-plus markers simultaneously, IMC enables identification of those markers critical to predicting response all in one image. Depicted here in pancreatic ductal adenocarcinoma: red: αSMA; cyan: E-cadherin; magenta: CD68; blue: iNOS; white: granzyme B; lime: collagen 1; yellow; HLA-DR

IMC captures the dynamic range of spatial biomarkers to enable predictive patient stratification

In a Phase 3 clinical trial[1] of trastuzumab for breast cancer in 180 patients, IMC enabled predictive patient response stratification by capturing the dynamic range of HER2 (high/low expression) and spatial proximity of the HER2 extracellular domain (ECD) and CD8+ T cells.

The ability to objectively measure HER2 domains, signaling targets and targeted immune cells using IMC delivers information with the potential to help identify patients who could benefit from targeted treatment or immunotherapy. For this study, the measurement of HER2 ECD and intracellular domain (ICD) in the same tissue and their association with better outcome was uniquely made possible by IMC.

Due to its high dynamic range, IMC identified the mechanism of action by which high expression of HER2 ECD attracts CD8+ cytotoxic T cells to the tumor site, facilitating the immune system's ability to target and eliminate cancer cells.

IMC provided a broader dynamic range versus quantitative immunofluorescence, allowing for more precise stratification of HER2 levels in clinical samples, as stated in the paper: "[quantitative immunofluorescence] has less dynamic range than IMC and appears to be saturated."

High and low HER2 expression detected by IMC reveals predictive patient response stratification

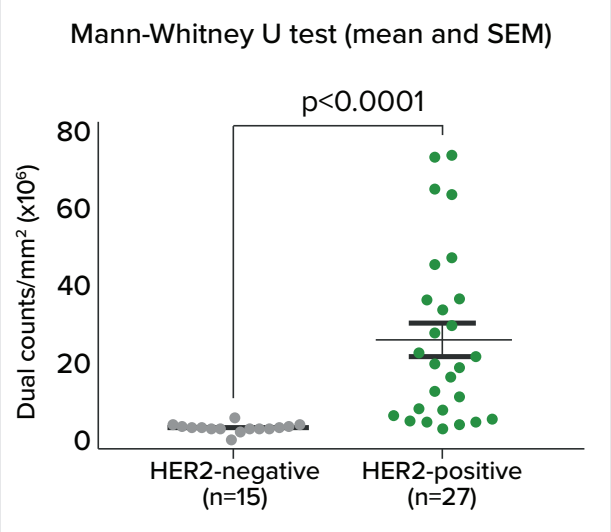

Figure 3. Dynamic range of HER2 expression captured by IMC is predictive of response. The study analyzed samples from HER2+positive breast cancer patients who were part of the HeCOG 10/05 clinical trial, captured in pixels on a standard tissue microarray (YTMA263).

Study at a glance

- A Phase 3 clinical trial evaluated whether breast cancer tumors that do not express the ECD of the HER2 protein are less likely to benefit from checkpoint therapy in 180 patients
- Due to high content coverage and high dynamic range capabilities provided by IMC, the study was able to determine outcomes only IMC could identify to successfully stratify patients
- IMC identified that high expression of HER2 and proximity to CD8+ T cells in the tumor microenvironment (TME) was indicative of response

IMC identifies pretreatment biomarker of response for checkpoint combination therapy

This study[2] analyzed pretreatment samples from 60 patients with metastatic melanoma treated with immune checkpoint inhibitors (pembrolizumab, nivolumab or ipilimumab plus nivolumab).

While immune checkpoint inhibitors have helped improve the median overall survival of metastatic melanoma, selecting the patients who will benefit prior to treatment would improve overall patient outcome and reduce challenges with adverse effects.

IMC identified 12 unique biomarkers of response, including B2M, MHC-1 and LAG-3, that were found to be associated with progression-free survival (PFS) and overall survival (OS).

The high dynamic range of IMC enables visualization of more markers and their expression levels on one slide, as further explained in the paper: "IMC data allowed us to quantitatively measure 25 markers simultaneously on formalin-fixed, paraffin-embedded tissue microarray samples."

IMC identifies predictive biomarkers of survival in checkpoint therapy

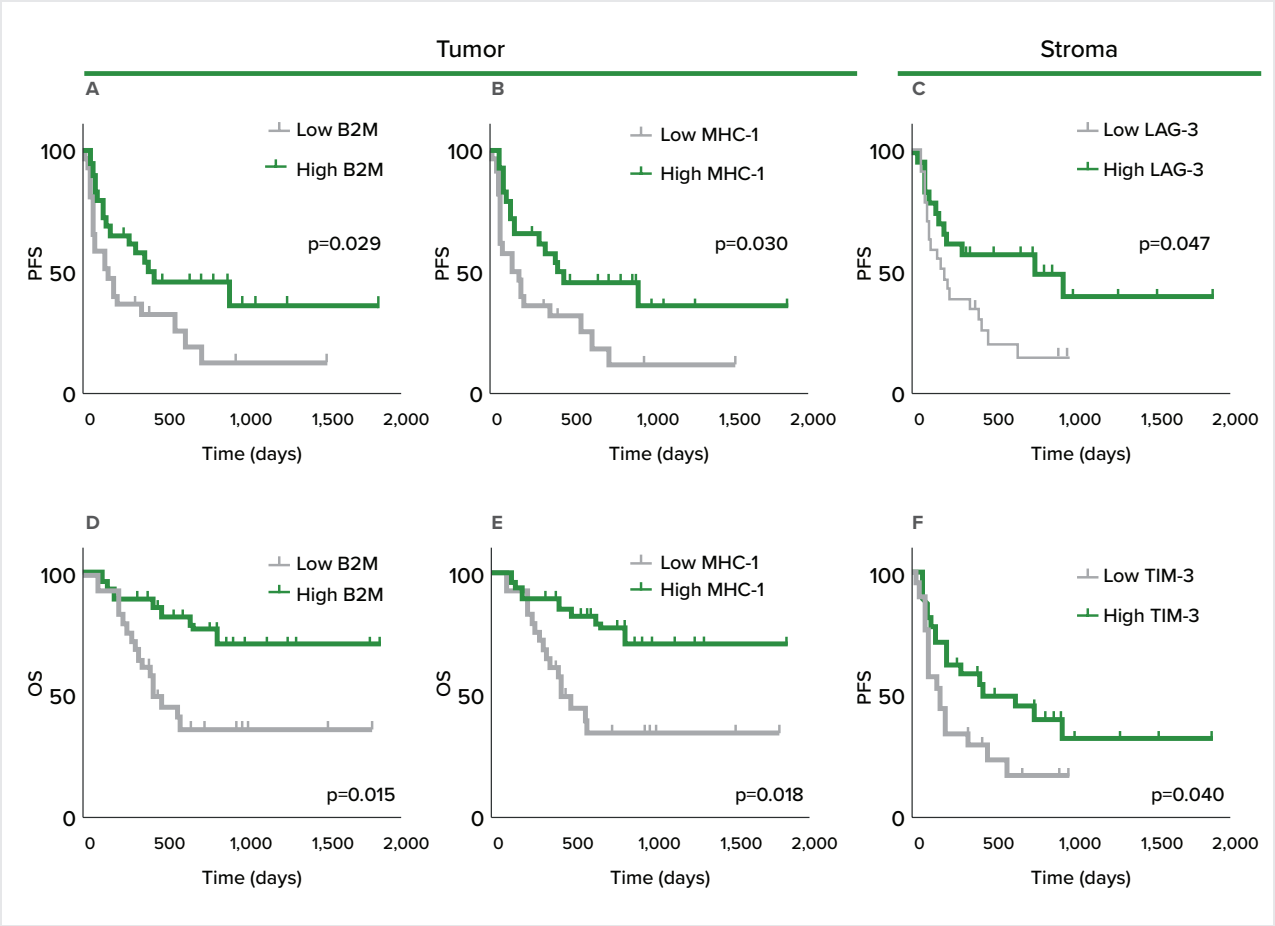

Figure 4. IMC data simultaneously measures 25 markers to identify predictive markers for survival for immunotherapy[2]. In Kaplan–Meier plots, high levels of B2M (A and D) and MHC-I (B and E) were associated with better PFS and OS, while melanoma patients with high levels of LAG-3 (C) and TIM-3 (F) showed better progression-free survival.

Study at a glance

- A 60-patient study tested the use of IMC as a selection tool to find patients who would benefit from immune checkpoint inhibitor therapy by measuring the expression of many variables simultaneously within the TME
- Spatial analysis using IMC enabled identification of a series of potentially indicative biomarkers of survival for immunotherapy in metastatic melanoma

IMC provides unique scale, dynamic range and throughput to uncover critical biomarkers missed by cyclic fluorescence

These case studies provide evidence of how IMC can better reveal mechanism of action, predictive response and relevant biomarkers over fluorescence imaging technologies across a variety of indications by:

- Capturing the dynamic range of spatial biomarkers to enable predictive patient stratification
- Identifying pretreatment biomarker of response for checkpoint combination therapy

Offering uniquely high dynamic range, IMC is the only solution that can deliver comprehensive data on expression levels in a highly reproducible, high-content fashion.

Summary

- IMC reveals the full range of protein expression, enabling immune insights unable to be captured with cyclic fluorescence
- By looking at up to 50-plus markers simultaneously, IMC detects therapeutic and immune responses that are critical to accurately determine safety and efficacy of a potential therapy, leveraging higher throughput and higher multiplexing without the need to manage autofluorescence.
- Examining protein expression levels in addition to presence or absence can better associate mechanisms to outcomes, enabled by IMC.
- The high dynamic range of IMC:
    - Uncovers spatial biomarkers that enable predictive patient stratification
    - Identifies pretreatment biomarker of response for checkpoint combination therapy

References

1. Carvajal-Hausdorf, D.E. et al. “Multiplexed (18-plex) measurement of signaling targets and cytotoxic T cells in trastuzumab-treated patients using Imaging Mass Cytometry.” *Clinical Cancer Research* 25 (2019): 3,054–3,062.
2. Martinez-Morilla, S. et al. “Biomarker discovery in patients with immunotherapy-treated melanoma with Imaging Mass Cytometry.” *Clinical Cancer Research* 27 (2021): 1,987–1,996.

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