Increasing Plexity of Imaging Mass Cytometry for Tumor Tissue Analysis

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Introduction

Increasing the number of investigated target markers on a single tissue enriches spatial characterization that facilitates a more accurate prediction of disease progression and preclinical outcome measures in clinical research projects using tumor biopsies or tissue microarrays (TMAs). Imaging Mass Cytometry™ (IMC™) is the leading platform for high-plex tissue imaging. IMC allows for detailed assessment of cell phenotype and function using 40-plus markers simultaneously at subcellular resolution on a single slide. A comprehensive IMC panel containing structural, functional, and immune markers enables us to reveal the complex heterogeneity of tumor tissues as well as the tumor microenvironment (TME). In addition to the need for a larger number of antibodies, there is an increasing demand for co-detection of mRNA markers on a single slide. Therefore, to increase the plexity of IMC panels, it is essential to expand the number of available metal channels. Here, we demonstrate the incorporation of conjugated antibodies with yttrium (89Y) and indium (115In), two low-mass metals, for IMC application. These metal tags have been previously tested as putative channels for IMC application

New Metals Allow for Expanded Easy Customization of the Human Immuno-Oncology IMC Panels

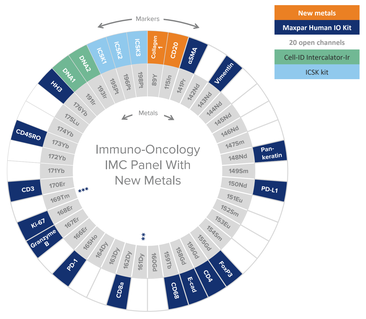

Figure 1. Addition of new metals to Human Immuno-Oncology IMC Panels. Panel above shows how the Maxpar® Human Immuno-Oncology IMC Panel Kit (Cat. No. 201508, **blue**), along with our Maxpar IMC Cell Segmentation Kit (ICSK, Cat. No. 201500, light blue), and Cell-ID™ Intercalator-Ir (Cat. No. 201192B, light green) can be used to easily customize and create new custom IMC panels. Two new metals (89Y and 115In, orange) were added to our catalog and allow for addition of new markers or replacement of existing Maxpar panel kit markers (as done in this poster, ** CD20-161Dy and *** Collagen-169Tm) in IMC panels. This panel has 20 open channels for customization with user-defined markers from our Maxpar and Maxpar OnDemand™ catalogs or custom conjugations.

Methods and Materials

We performed conjugation of antibodies to 89Y or 115In to expand the number of channels available for IMC analysis. Conjugations were done at multiple scales. IMC analysis of various tissue types stained with panels of conjugated antibodies, including the novel 89Y- and 115In-conjugated antibodies, was performed. We compared images for the 89Y- and 115In-conjugated antibodies with the images generated using Maxpar catalog antibodies of the same clones, with a focus on marker specificity and background signal.

Imaging Mass Cytometry workflow

1 Design
Design panels using Maxpar and Maxpar OnDemand antibodies conjugated to metal tags.

2 Stain
Stain formalin-fixed, paraffin-embedded (FFPE) tissues using familiar immunohistochemistry (IHC) protocols.

3 Image
Image protein markers at subcellular resolution using the Hyperion™ Imaging System.

4 Analyze
Analyze images in minutes using MCD™ Viewer and easily export for secondary analysis.

Figure 2. Imaging Mass Cytometry workflow. The full panel was applied on mouse normal and tumor TMA containing a wide variety of tissue types. Stained tissues were ablated using the Hyperion Imaging System at 200 Hz with 1 µm pixel size. Resulting images were rendered in MCD Viewer and exported for single-cell analysis. All antibodies were titrated and tested on positive control tissue (spleen, colon, lung) alongside tumor samples. For additional details, please see application note accessible through the QR code below.

Results

Our results open a new avenue to assign markers to 89Y and 115In, which enables a larger list of potential targets to be investigated in any IMC study. Expanding the number of markers to 40-plus in Imaging Mass Cytometry will improve the imaging results necessary to identify novel cell signatures (phenotype and interactions) in the TME. The 89Y- and 115In-conjugated antibodies showed equivalent specificity and staining quality compared with the existing lanthanide-conjugated Maxpar catalog antibodies of the same clone on serial sections (Figure 3). IMC analysis of various tissue types stained with panels of conjugated antibodies, including the novel 89Y- and 115In-conjugated antibodies, showed marker specificity and low background signal (Figures 3–9), including in autofluorescent tissues like colon adenocarcinoma (Figure 7) and liver hepatocellular carcinoma (Figure 8).

Comparison of New Metal Conjugates to Existing Maxpar Lanthanide Conjugates

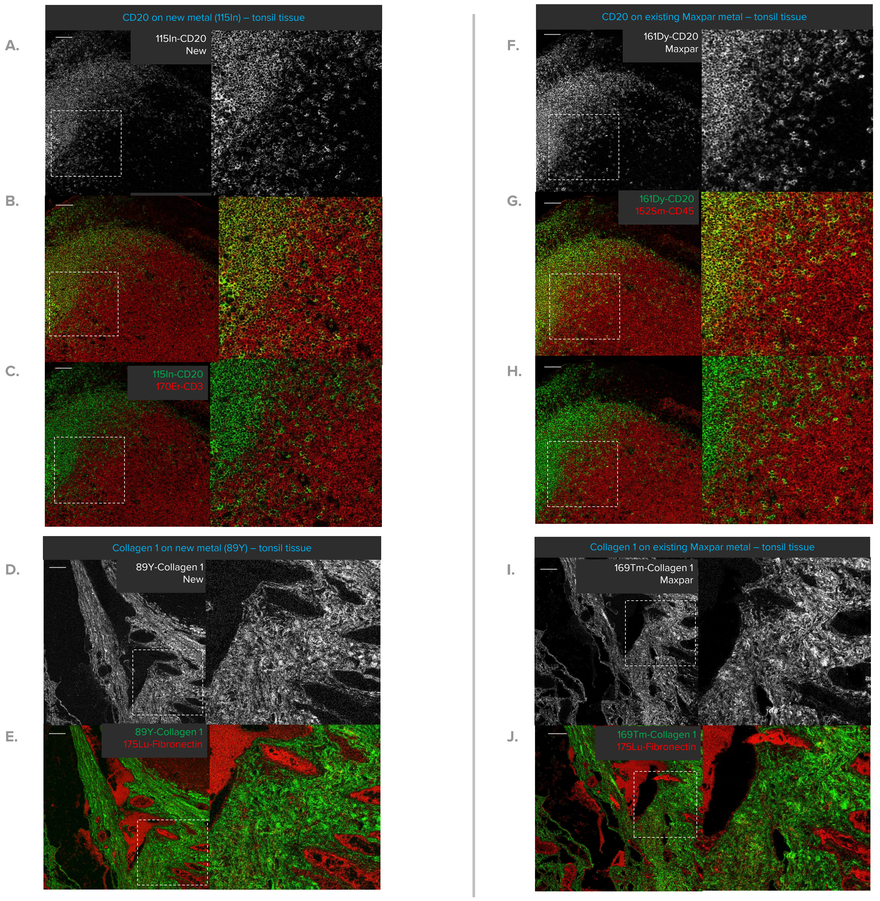

Figure 3. Conjugates with new metals compare to existing Maxpar products in IMC applications. Human tonsil was stained with an Immuno-Oncology Panel including new metal conjugates **(A–E)** or Maxpar antibodies **(F–J)**. **A.** Anti-CD20 antibody (clone H1) conjugated to **(A)** 115In or **(F)** Maxpar antibody (161Dy). (B) CD20 co-staining with CD45 for the new metal (115In) or **(G)** Maxpar antibody (161Dy). **(C)** CD20 counter-staining with CD3 for the new metal (115In) or **(H)** Maxpar antibody (161Dy). **(D)** Anti-collagen type 1 (polyclonal) antibody conjugated to 89Y or **(I)** Maxpar antibody (169Tm). **(E)** Collagen 1 counter-staining with fibronectin for new antibody conjugate to (89Y) les or **(J)** Maxpar antibody (161Dy). Images were generated in MCD Viewer (Standard BioTools) with maximum threshold >10, minimum threshold = 0, and gamma = 1 for both new metals and corresponding Maxpar controls. Scale bar = 100 µm

Highlighting IMC Staining of New Metal Conjugates on Various Tissues

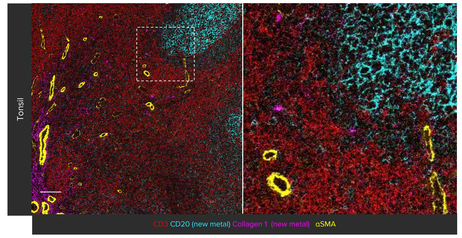

Figure 4. IMC Staining of tonsil. Normal human tonsil (left) and inset (right) shows presence of B cells (CD20) in the germinal center,and T cells (CD3) in surrounding non-germinal center. Presence of alpha-smooth muscle actin (αSMA) lining vessels and collagen 1 (ECM components) expressing stromal cells are detected. Scale bars = 100 µm

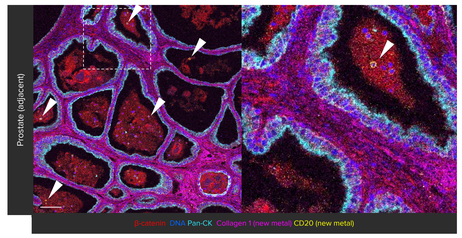

Figure 5. IMC staining of prostate. Human prostate (adjacent, left) and inset (right) shows presence of isolate B cells (CD20+, arrowheads) in glandular tissue (β-catenin+). Epithelial tissue is identified by pan-cytokeratin staining. Presence of collagen 1 (ECM components) expressing stromal cells are detected. Scale bars = 100 µm

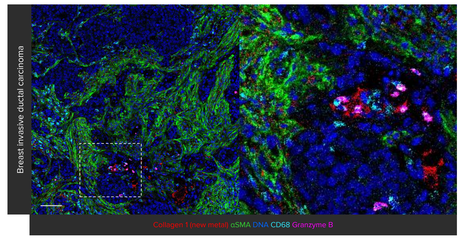

Figure 6. IMC staining of breast invasive ductal carcinoma. Staining of breast invasive ductal carcinoma (left) and inset (right) shows presence of αSMA and collagen 1 (ECM components) expressing stromal cells. Presence of macrophages (CD68+) and granzyme B expressing cells including macrophages can also be detected in this tissue. Scale bars = 100 µm

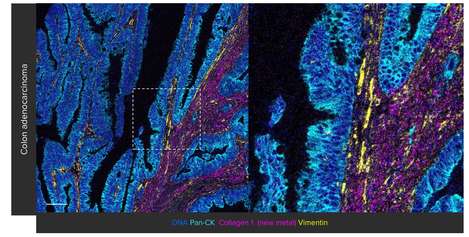

Figure 7. IMC staining of colon adenocarcinoma. Staining of colon adenocarcinoma (AC, left) and inset (right) shows presence of vimentin and collagen 1 (ECM components) expressing stromal cells. Tumor tissue is identified by pan-CK staining. CD20+ cells (B cells) were not detected in this tissue. Scale bars = 100 µm

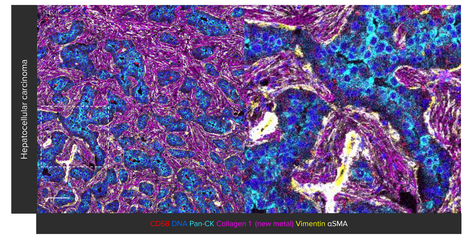

Figure 8. IMC staining of hepatocellular carcinoma Staining of hepatocellular carcinoma (HCC, inset right) shows presence of αSMA, vimentin and collagen 1 (ECM components) expressing stromal cells. Tumor tissue is identified by pan-CK staining. Macrophages (CD68+ cells) can be seen infiltrating the tumor. CD20+ cells (B cells) were not detected in this tissue. Scale bars = 100 µm

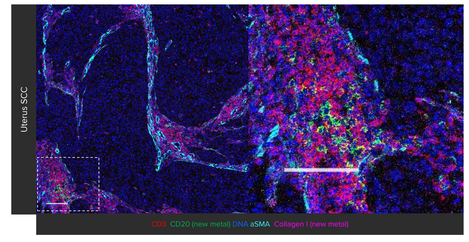

Figure 9. IMC staining of uterus squamous cell carcinoma. Left: Immune cell infiltration in uterus squamous cell carcinoma (SCC) clusters of T cells (CD3+) and B cells (CD20+) is observed. Collagen 1 and αSMA (ECM components) expressing stromal cells are also detected. Right: inset. Scale bars = 100 µm

Conclusions

- New channels allow marker assignment to 89Y and 115In metals, opening new research avenues.
- 89Y- and 115In-conjugated antibodies showed equivalent specificity and staining quality compared with the lanthanide-conjugated catalog antibodies of the same clone.
- Moving existing markers to new 89Y and 115In metals (161Dy and 169Tm in this poster) opens 2 existing channels for additional flexibility in user-defined markers.
- Expanding the number of channels/markers to 40-plus in Imaging Mass Cytometry will improve the imaging results necessary to identify novel cell signatures (phenotype and interactions) in the TME.
- More channels allow users to answer more questions with protein markers or RNA (other applications).

❖ **New metals/conjugates are available through Standard BioTools™ Custom Conjugation Services – see our website (QR code below) or contact Field Application Scientists (FAS) or Technical Support Specialists (TSS) for details.**

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