Comparison of Multiplexed Imaging Mass Cytometry with Monoplex Immunohistochemistry in FFPE Tissue

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Abstract

Multiplexed analysis of limited tissue samples can improve our understanding of tumor biology and the tumor microenvironment. Chromogenic and fluorescent multiplexed immunohistochemistry (IHC) approaches are available and offer great insights while conserving limited tissue; however, these approaches have their limitations. Multiplexed chromogenic IHC methods can at best accommodate up to 3 distinct markers. Fluorescence-based approaches can support a higher degree of multiplexing; however, spectral overlap issues and differences in labeling efficiency and photostability complicate experimental procedures and data interpretation. Imaging mass cytometry™ (IMC) has recently emerged as a novel technology for tissue imaging that enables multiplexed analysis of protein expression (up to 40 markers) in a single tissue sample while circumventing the limitations of chromogenic and fluorescent IHC techniques. Metal-conjugated antibodies are used to perform qualitative and quantitative analyses of the expression of multiple proteins of interest on a single formalin-fixed, paraffin-embedded (FFPE) tissue slide. Here we compare the performance of the IMC method with that of conventional, established IHC techniques using a small panel of markers.

Methods

Multiplex CyTOF-IMC Staining With Metal-Conjugated Antibodies

Non–small cell lung cancer (NSCLC) and tonsil FFPE tissue sections (4 µm) on glass slides were baked at 60°C for 2 hours. After deparaffinization, antigen retrieval was performed in 100 mL of 1x Citra Plus buffer (pH 6.0, Biogenex Cat #HK080-5K) at 121°C for 20 minutes. Tissue sections were blocked using 500 µL of 3% BSA/0.1% Triton X-100/PBS at room temperature for 45 minutes. After being blocked, tissue sections were incubated in 300-µL metal-conjugated cocktail antibodies (Table 1) at 4°C overnight. Stained tissue sections were analyzed using CyTOF-IMC, which consists of a laser ablation unit (Hyperion™) coupled with a mass cytometer (Helios™). Stained slides were air-dried and loaded into the ablation chamber. The regions of interest (ROIs) for ablation were identified based on the IHC staining of sequential sections (see below). Each ROI was set to 2000x2000 µm and ablated using a 1-µm laser beam at a frequency of 200 Hz. The ablated plumes (1 μm^2) were transferred into the Helios system for the quantification of isotope abundancy per plume. The data were generated in an MCD format using version 6.7.1014 of the CyTOF software. One tissue section was multiplexed with 5 different metal-conjugated antibodies together. For the IHC comparison (see below), each antibody was stained as a monoplex.

Monoplex IHC

Serial sections from single blocks of archival NSCLC and tonsil FFPE human tissue were used in this study. Sections were cut at 4 µm and mounted on positively charged glass slides. The slides were baked at 60°C for 60 minutes and placed into the Leica BOND RX autostainer. Automated IHC staining was conducted for monoplex staining of Programmed Death Ligand-1 (PD-L1; Ventana M4420), CD8 (Abcam ab75129) and CD68 (Abcam ab995), with antibodies diluted in Leica Antibody Diluent (#AR9352). Tissue image analysis using Flagship's proprietary cTA® platform was used for the classification and quantification of the staining intensity of each chromogen in each cell identified by the algorithm. Once the cells were correctly identified, staining intensity thresholds were defined to quantify chromogen intensity and accurately classify cells based on the percentage of positive cells relative to the total number of cells.

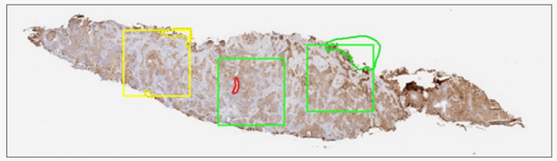
TABLE 1. Description of Antibodies Used for IMC and IHC Experiment

		IMC				IHC		
Antibody	Clone	Metal (IMC)	Vendor	Catalog #	Final Conc.	Vendor	Catalog #	Final Conc.
PD-L1	SP142	169Tm	Spring Bio	M4420	7 µg/mL	Ventana	M4420	7 µg/mL
CD8	C8/144B	160Gd	LS Bio	LS-C311966	1 µg/mL	abcam	ab75129	1 µg/mL
CD68	KP1	159Tb	Abcam	ab995	2 µg/mL	abcam	ab955	2 µg/mL

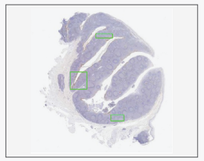
Selection of Comparative ROI's

While Flagship's cTA platform is capable of digital imaging of the whole slide region, IMC technology requires the selection of ROIs for ablation. For the comparative studies, 3 ROIs were selected, as highlighted by the colored yellow and green squares below. The 3 ROIs were ablated in CyTOF-IMC, and signals were collected and stored as mcd files. For IHC, similar ROIs were selected and analyzed using Flagship's cTA platform.

NSCLC ROIs

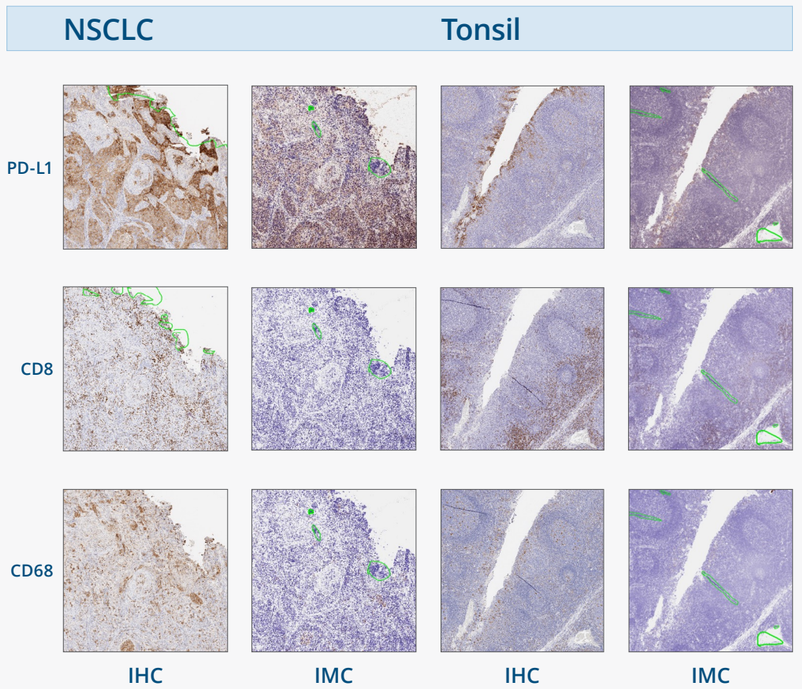


Tonsil ROIs



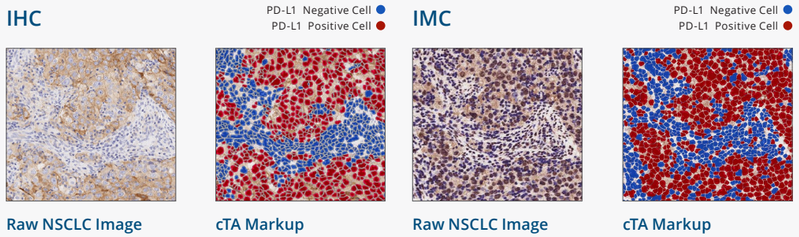
IMC vs IHC

NSCLC and tonsil serial sections were subjected to multiplexed IMC and monoplex IHC. Comparative ROIs were selected for digital image analysis. The images below are representative ROIs for each biomarker in the NSCLC and tonsil tissues. Green indicates an area of exclusion. While there was a visible decrease in intensity, the distribution patterns and biomarker quantifications remained similar between sections analyzed with IHC and those analyzed by IMC.



The cTA Platform

The use of cTA-based scoring as opposed to manual scoring allows for direct cell counting and scoring across the whole slide. The cTA markup is a visual representation of the data generated by the algorithm. The images below represent the raw images and cTA markup of a PD-L1–stained NSCLC slides.



Comparative Analysis

The staining patterns of the corresponding biomarkers demonstrated similar distribution patterns between IMC and IHC on sequential sections. A digital image analysis using Flagship's cTA platform demonstrated concordance in the percentage of biomarker-positive cells within the analyzed matched IHC and IMC areas (Table 2). The percentages were averaged across the 3 analyzed ROIs. It was also demonstrated that cellular image segmentation can be performed on IMC images, thus allowing for the use of various software packages for high-dimensional single-cell analysis of IMC data.

TABLE 2. Average Percent Positive Biomarker Expression in NSCLC and Tonsil Sections Subjected to IHC and IMC

		BIOMARKER		
		PD-L1	CD8	CD68
NSCLC	IHC	46.82	25.58	18.40
	IMC	51.66	33.11	12.85
Tonsil	IHC	11.67	10.24	4.03
	IMC	15.73	9.57	4.92

Conclusion

- Digital image analysis data generated using Flagship's cTA platform on FFPE tissue samples subjected to multiplexed IMC platform correlate with data generated from FFPE tissue samples subjected to monoplex IHC.
- The IMC platform is a new tool capable of reliably and dramatically enhancing our ability to study the biology of cancer using a highly multiplexed analysis of limited tissue samples, but it requires companion quantification tools.