A novel cross-site analysis of Vectra® Polaris™ multiplex fluorescence PD-1/PD-L1 immunohistochemistry on colorectal cancer with high and low microsatellite instability

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Introduction

Background: Colorectal cancer (CRC) is the third most diagnosed cancer in the United States with a projected 52,980 deaths in 2021 [1]. Microsatellite instability-high (MSI-H) CRCs with deficiencies in mismatch repair (MMR) are significantly associated with positive response to immunotherapy and improved outcomes when treated with immune checkpoint inhibitors [2,3]. Programmed cell death ligand-1 (PD-L1) is an effective biomarker of MSI-H status to identify CRC patients who will respond to treatment, however, reproducible quantification of programmed cell death receptor-1 (PD-1)/PD-L1 in the tumor microenvironment (TME) across laboratory sites has been under-reported. In this study, our group directly addressed this issue by interrogating PD-1/PD-L1 expression cross-site at Akoya Biosciences and NeoGenomics Laboratories by employing the MOTiF™ PD-1/PD-L1 Panel kit along with the Vectra Polaris imaging system.

Methods: Serial sections from 40 CRC samples with known MSI status were stained at Akoya and NeoGenomics Laboratories using a modified MOTiF PD-1/PD-L1 Lung Panel Kit on the Leica BOND RX. Sections were scanned using the Vectra Polaris imaging system at both sites. Cross-imaging and intersite staining reproducibility was assessed using image analysis algorithms developed with inForm tissue analysis software. Cell counts and densities were calculated using the R-script package PhenoptrReports and correlations were plotted per marker.

Results: The average signal intensity for all markers/Opal fluorophores was within the recommended ranges of 10-30 normalized counts, with the exception of Polaris 780, which has an advised range of 1-10. This indicates the protocol stained successfully and reproducibly across all serial sections at both sites. Cross-instrument concordance analysis of cell densities for each marker yielded an average R^2 value of 0.95.

Conclusions: This study demonstrated that the multiplexed fluorescence (mIF) PD-1/PD-L1 Panel kit imaged in conjunction with the Vectra Polaris is not only a reliable assay that can be run across different sites, based on the concordant cross-instrument and intersite staining data, but that re-optimization of the kit allows for the assay panel to be successfully adapted to other cancers, such as CRC, which can then capture biological differences across a multitude of samples.

1. American Cancer Society https://www.cancer.org/cancer/colon-rectal-cancer/about/key-statistics.html
2. Yi M, Jiao D, Xu H, Liu Q, Zhao W, Han X, et al. Biomarkers for predicting efficacy of PD-1/PD-L1 inhibitors. Mol Cancer. 2018;17(1):129
3. Lemery S, Keegan P, Pazdur R. First FDA approval agnostic of cancer site - when a biomarker defines the indication. N Engl J Med. 2017;377(15):1409–12.

Overview of Study Workflow

Figure 1. Assay Workflow. The Akoya MOTiF PD-1/ PD-L1 Panel kit was adapted and optimized to work on CRC. Serial sections from 40 CRC samples were distributed to both sites and stained using the modified protocol on the Leica Bond RX autostainer. Whole slide multispectral images of the stained slides were acquired on both Polaris at NeoGenomics and Akoya. Five matching regions of interest were selected across serial sections and a locked down inForm algorithm was developed. Images underwent tissue segmentation, cell segmentation, phenotyping, and thresholding. Data exported from inForm was processed in phenoptrReports and both Polaris platform and staining inter-site concordance was assessed.

Key Findings

In this study, 40 CRC samples were analyzed using a modified MOTiF PD-1/PD-L1 kit at Akoya and NeoGenomics Laboratories. The staining was performed on the Leica Bond RX autostainer and imaging was acquired using Vectra Polaris. Cross-imaging and intersite staining reproducibility was assessed. Overall, robust concordance was achieved.

- An average Polaris platform concordance of R^2 =0.95 was achieved for cross-imaging reproducibility across all biomarkers in the assay.
- Inter-site staining assessment showed an average concordance of R^2 =0.81. Five of the six markers showed strong concordance with an average R^2 of 0.87, with the exception of CD68. More evaluation is underway to explore how to improve CD68 concordance. Macrophages represent a specific image analysis challenge due to their variation in size, morphology and staining pattern.

Representative Biomarker Staining in CRC using MOTiF PD-1/PD-L1 kit

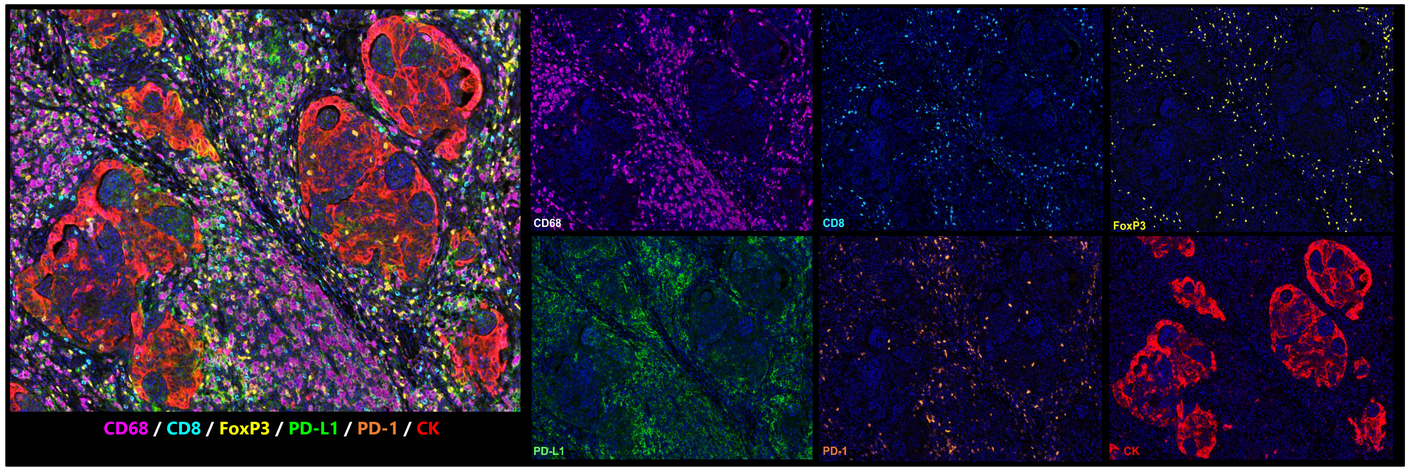

Figure 2. Representative multiplex immunofluorescence staining using the modified MOTiF PD-1/PD-L1 Lung Cancer kit on CRC samples. A composite image and individual channels representing each of the six markers in the kit. Images acquired using the Vectra Polaris. Magenta = CD68 (Opal Polaris 780), Cyan = CD8 (Opal Polaris 480), Yellow = FoxP3 (Opal 570), Green = PD-L1 (Opal 520), Orange = PD-1 (Opal 620), and Red = CK (Opal 690).

MOTiF Kit 6-plex mIF Panel

Order	Opal	Marker [clone]	Top 20 (%CV)
1	570	FoxP3 [D608R]	18 (6%)
2	520	PD-L1 [E1L3N]	20 (9%)
3	690	CK [AE1/AE3]	15 (2%)
4	620	PD-1 [EPR4877] [EPR4877]	19 (4%)
5	480	CD8 [4B11]	11 (9%)
6	780	CD68 [PG-M1]	7 (16%)

Table 1. MOTiF Kit Staining Parameters and %CV. Staining order and Opal-marker pairings were maintained during the adaption of the Lung Cancer kit to CRC samples. The mean expression for the top 20 cells was calculated for each marker across positive control CRC slides. Average Top 20 signal intensity counts fell within the recommended Opal counts guidelines of 10-30, with the exception of Opal Polaris 780, which has a recommended count of 1-10. Average % CV was <10%, suggestive of excellent reproducibility except for CD68, which is known to display more variability due to its staining pattern.

Concordance Analysis

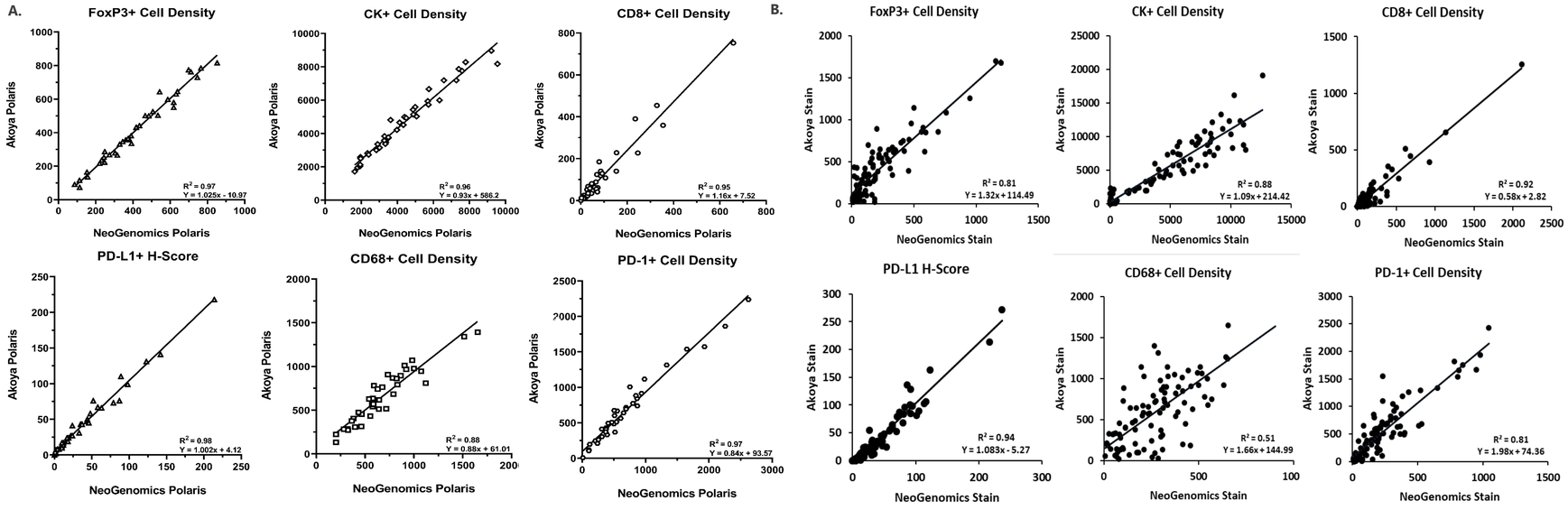

Figure 3. Cross-Polaris and intersite staining reproducibility concordance of cellular markers. A. Concordance plots showed strong Polaris platform concordance for all the biomarkers in the panel, with an average R^2 for all phenotyped markers of 0.95. Importantly, PD-L1 and PD-1 across Polaris platforms displayed the highest concordance at 0.98 and 0.97, respectively. Graphs represent concordance analysis between cell densities for each marker on Vectra Polaris imaging systems at both sites. **B**. Once Polaris cross-site concordance was established, inter-site staining reproducibility was assessed using the same algorithm across stained slides. Excellent concordance was observed for five markers which displayed R^2 values of 0.81 or greater, with the exception of CD68, whose score was 0.51. In particular, the PD-L1 score demonstrated the greatest concordance with R^2 values of 0.94, indicating consistent reproducibility across both sites for staining and imaging. Excluding CD68, the average R^2 for all markers for inter-site staining reproducibility was 0.87.