Multiparametric Immunohistochemistry Quantification With Computational Tissue Analysis for Tumor Immune Profiling

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Abstract
Understanding the patients’ tumor immune profile is key to providing the most effective immunotherapy treatment strategy. Multiplex immunohistochemistry (IHC) combines multiple biomarker assays on a single tissue section to examine multiple cell types or biological pathways that might be used in a single or combination therapeutic strategy. Notably, multiplex IHC assays allow for the assessment of end points that measure the spatial relationships between cell types expressing certain biomarkers, such as the colocalization of biomarker or distance measurements between multiple biomarker-expressing cells of interest. Flagship’s Computational Tissue Analysis (cTA™) platform allows for the quantification of individual analytes on dual-stained tissue sections, enabling the investigation of complex biological questions of spatial relationships and biomarker colocalization that cannot be achieved with traditional tissue-based manual end points.

Analytical Validation of the CD8/Ki67 Duplex IHC-cTA™-Based Assay in NSCLC

A multiplex IHC assay allows for the quantification of each biomarker in the tumor compartment and tumor microenvironment range, analytical sensitivity, analytical specificity, accuracy, and precision. The percentage of cells positive for Ki67 nuclear staining or CD8 IHC assays, this study utilized a reference method and multiple days of staining to assess 5 performance criteria for the assay: reportable range, analytical sensitivity, analytical specificity, accuracy, and precision. The percentage of cells positive for Ki67 nuclear staining or CD8 membrane staining was quantified using the cTA™ platform. Performance of the chromosome: IHC-cTA assay-based in quantifying Ki67 and CD8 was considered acceptable for the 5 criteria evaluated. Once the performance of the assay was established, additional exploratory cTA-based end points were examined, including the quantification of each biomarker in the tumor compartment and tumor microenvironment and analysis of the spatial arrangement of immune cells relative to tumor cells.

Analytical Sensitivity and Specificity

The reportable range in the duplex IHC-cTA assay was approximately 0% to 30% for Ki67-positive cells and 0% to 45% for CD8-positive cells.

Accuracy
Three components were used as a bridge to validate the primary end points for the Ki67/CD8 duplex IHC-cTA assay: 1. Reference assay: previously validated monoplex assay scored with a manual pathology approach 2. Monoplex assay: monoplex assay (using the same chromogen as in the duplex assay for each biomarker) scored with cTA-based end points 3. Duplex assay: duplex assay scored with cTA-based end points

Reference Assay – Monoplex Assay
• The Pearson correlation coefficients (PCC) between the reference assay and the monoplex assay were above the predefined performance specification for both biomarkers (Ki67 = 0.79; CD8 = 0.97).
• The monoplex assay was qualified as an acceptable reference standard for evaluating the duplex assay.

Monoplex Assay – Duplex Assay
A strong correlation between the monoplex assay and duplex assay results was observed.

CD8 Monoplex to Duplex Correlation
PCC = 0.96

Ki67 Monoplex to Duplex Correlation
PCC = 0.82

Conclusions
The use of a cTA™-based approach allows for:
• The reporting of accurate and precise tissue biomarker measurements with a high-complexity staining multiplex IHC assay
• The quantification of multiple biomarker parameters in the tumor and TME compartments
• The measuring of spatial arrangements can reveal context-related biomarker expression patterns for tumor immune profiling

The cTA™ platform demonstrated differential expression of activated T cells between the tumor and TME.

In this study, the distance between a CD8+ or Ki67+/CD8+ cell in the TME and the nearest Ki67+ tumor cell was measured. The probability density curve plots the number of CD8+ cells in the TME at a given distance (measured in µm) from the nearest Ki67+ tumor cell.

Examples NSCLC 42 and NSCLC 49 display similar values of percent positive cells in the tumor and TME, while NSCLC 42 is displayed significantly higher values. The fraction of Ki67+/CD8+ cells within 25 µm of the Ki67+ cell is used as an end point, the spatial patterns of NSCLC 49 and NSCLC 42 are observed to be more similar than for NSCLC 42, which has a higher fraction of cells in close proximity. The combination of these spatial values can provide context for understanding the interaction of these cell types. In the case of Ki67+/CD8+ cells, the proximity to dividing Ki67+ tumor cells may indicate a greater potential for tumor cell death.