Providing Confidence Around Computational Tissue Analysis Using Heterogeneity Assessments

Carsten Schnatwinkel, Famke Aeffner, Daniel Rudmann, Will Paces, Jasmeet Bajwa, Michael Sharp, Gerry Chu, Natalie Hutnick, J. D. Alvarez

Abstract

Background: Although the techniques to interrogate the appearance of a biomarker in tissue sections have greatly advanced, there are limitations as to how representative an analysis of a tissue section is compared with that of the entire diseased tissue. Depending on the heterogeneous expression level of a biomarker, tissue sampling can result in different interpretations of the biomarker’s appearance and hence could lead to a false result regarding the biomarker’s presence. Such inaccurate data, of course, could result in ineffective patient care decisions.

Hypothesis: Digital image analysis has demonstrated tremendous value in quantifying many features related to biomarker distribution and expression in biological tissues. The interpretation can be collected for various indications and biomarkers, and a phenotypic signature can be established that describes a biomarker representation across indications. Moreover, the assessment of new samples can be compared with the established phenotypic signature, and a confidence designation can be applied in support of the determined end point.

Approach: For a proof of concept, 6 prostate cancer samples were processed, and a single section was collected after every 100 µm. A total of 7 sections per sample were stained for the lymphocyte marker CD3, and the number of positive target cells was determined in the tumor using Computational Tissue Analysis (cTA™). To assess how indicative the evaluation of a single tissue section would be for the entire tumor, the heterogeneity level was determined on the section level as well as by random grid analysis on each individual section. Both criteria were utilized to define an indication- and biomarker-specific confidence score.

Conclusions: The combination of immunohistochemistry (IHC) and cTA™ is a powerful tool to convert complex data into meaningful interpretations. cTA™ is also capable of cataloguing and the greater variability in smaller biopsies.

Data Collection

The immune infiltrate is an important component when evaluating the response of an immunomodulatory therapy. Beyond the identification of appropriate immune biomarkers, there is the challenge of spatial heterogeneity of immune cells across biopsy samples as the interpretation. Here Flagship uses CD3 as a marker for assessing T-cell infiltrates in prostate cancer and describes a potential path of how a spatial heterogeneity assessment can assist in providing confidence around biomarker interpretation.

Flagship hypothesizes that the spatial heterogeneity level of CD3 across multiple sections is similar to the level of spatial heterogeneity across multiple regions of interest (ROIs) within a single section (see schematic illustration below). If true, a spatial heterogeneity assessment of a single section would allow the biomarker evaluator to determine whether the information obtained from a single section is close to a true representation of T-cell infiltrates across the entire tumor.

Tissue Image Analysis

All CD3-stained sections were annotated to capture the core tumor tissue. As illustrated below, there are intrinsic challenges that may prevent consistent assessments across sections for a given sample (eg, areas that do not reveal tumor content and hence would not be included in the analysis region). The figure below also highlights the difference between immune infiltrates in regions with high tumor content (low infiltrate) and those in regions with low tumor content (high infiltrate). Algorithms were designed to capture DAB-positive CD3 cells in the entire tissue sections as outlined below. No tumor/stroma separation was performed at this point.

Building a Reference Library

The data set from Flagship’s cTA™ approach was used to establish the confidence interval (macroheterogeneity) for the assessed biological variability of the percentage of CD3-positive cells in the whole sections or defined ROIs (1-mm² boxes or 0.25-mm² boxes).

Evaluating Acceptance Criteria

The box plot is illustrating the distribution of the percentage of CD3-positive cells for 2 prostate cancer patient samples collected from 7 sections that were 100 µm apart. Sample CNT0T64 represents the range of percent positive CD3 staining across different ROIs as well as the IQR indicate an increased level of heterogeneity compared with the range seen across 7 sections (above). This is expected based on the variability seen when smaller ROIs are utilized. With the introduction of the Prostate Cancer Reference Library for CD3, sample CNT0T64 would disqualify for a confident representation of the tumor biology due to having less than 3.5% positive cells. In addition, based on the broad IQR and the greater-than-0.8 CV, sections 1 through 6 may indicate a lack of confidence score. This result is recapitulated by the fact that none of the section’s individual score is close to the total percentage of CD3-positive cells (10.5%) for this patient.

For CNT0T64, the range of percent positive CD3 staining across different ROIs as well as the IQR indicate an increased level of heterogeneity compared with the range seen across 7 sections (above). The level, however, is smaller than that of CNT0T64. The analysis sections results in percent positive CVs for CD3 that are within the determined interval in the established Prostate Cancer Reference Library. In addition, the narrow IQR and lower CVs (>0.6) for sections 2, 3, and 7 would indicate a high confidence score. The remaining sections would obtain a medium confidence score. With the exception of section 1, all samples are close to the total score (8.4%) for this patient sample.

Applying a Method

• The percentage of lymphocytes in low versus high tumor high content areas can bias data interpretation.
• There is intratissue variability for % positive CD3 evaluation in patient samples.
• Variability can be caused by
  • Biological diversity.
  • The absence of 3-dimensional information.
  • The impact of intratissue variability seems to be more sensitive to samples with overall low CD3+ cell counts.
• Lymphocyte hot spots (eg, tertiary follicles) can cause potential misinterpretations.
• A separate tumor/stroma evaluation may provide additional confidence in the assessment.
• Tissue parameters that would allow for a more robust and representative assessment can be defined and included in the Prostate Cancer Reference Library (eg, the minimum content of tumor vs stroma).
• The Prostate Cancer Reference Library is a living document. It can be updated with additional sample analyses and based on therapeutic interventions.

Distribution of % CD3-Positive Cells Across 7 Sections

<table>
<thead>
<tr>
<th>Section</th>
<th>Whole Sections</th>
<th>1-mm² Boxes</th>
<th>0.25-mm² Boxes</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNT0TAA</td>
<td>Low Low Low Low Low Low Low</td>
<td>Medium Medium Medium Medium Medium Medium Medium</td>
<td></td>
</tr>
<tr>
<td>CNT0T64</td>
<td>High High High High High High High</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Poster #: 1710