Impact of tissue processing and interferents on the reproducibility and robustness of a multigene expression assay measuring tumor inflammation.

NanoString Technologies, Seattle, WA, USA

Abstract # 4244

The Tissue Inflammation Signature (TIS) is an investigational use RNA expression assay on the NanoString nCounter® Dx Analysis System, which provides a measure of tumor inflammation across multiple solid tumor types. TIS measures immune genes in tumors from multiple origins, and its ability to include tissue-specific interferents, such as non-tumor lymphoid aggregates (NTLA) could influence TIS performance. Here we describe the validation of the reproducibility of the TIS assay starting from FFPE tissues and robustness of the TIS across 8 potential tissue interferents.

Overview of TIS Algorithm & Test

- The TIS-gene signature was derived by a cross-validated penalized regression for response in a permutation-tiled survival in 125 patients from the KEYNOTE-012 trial (biliary, esophageal, gastric, gastroesophageal reflux disease, head and neck, lung, mesothelioma, pancreatic, salivary, cervical, endometrial, vulvar) cohorts. On average, the TIS-gene signature was derived using 25.1% of the tissue by area.
- The TIS-gene signature consists of 18 biomarker and 10 normalizer genes.
- RNA is analyzed using the NanoString nCounter® TIS measures gene expression counts of 18 biomarker and 10 normalizer genes.
- Gene counts from RNA are used to derive a score which is converted to a biomarker category to inform on treatment decisions. The score could then (or could potentially) benefit from anti-PD1 therapy. TIS is being developed for decentralized testing across multiple facilities and services.
- The precision of the test and biomarker category when reproducibly measuring the same RNA sample were previously validated across multiple laboratories.

Reproducibility of TIS from FFPE Tissue

- Why test for reproducibility?
  - TIS assay requires pathologists to review H&E slides to identify tumor content which lab technicians provide in a database.
  - The H&E review and tissue processing could cause variability in TIS score and biomarker classification.

- Why test for interferents?
  - Tissue interferents are represented as substrates encoded previously or present within and adjacent to tumor tissue that could cause an aberrant assay result.
  - It has been tested whether multiple different potential interferents in the TIS.
  - Non-specifics tumor interferents were examined for tumor types.
  - Specific tissue interferents (TIS) for each tumor type were also tested following recommendation by multiple pathologists.

- Study Design Overview
  - 11 tumor types and 22 independent pooled FFPE samples (170 total FFPE samples tested) were analyzed.
  - 3-parallel 3 replicates independently marked HA from the same tumor tissue
  - 3-parallel 3 replicates were performed on the RNA samples
  - All RNA processing on the same TIS assay
  - Parallel 3 replicates (3 tests per sample) for concordance

- Results for Tissue Reproducibility
  - TIS assay chain of custody
  - 96% of samples passed QC
  - Scores are reproducible across multiple pathologists and assays through the same tissue (Figure 2).
  - The assay is scalable, a 1:10 sample to assay ratio is expected.
  - The TIS assay requires pathologists to review H&E slides to identify tumor content which lab technicians provide in a database. The H&E review and tissue processing could cause variability in TIS score and biomarker classification.

- Results for Tissue Interferents Studies
  - This TIS assay procedure requires macrodissection if the tumor area covered by the pathology on the H&E is > 50% of the total tissue area.
  - 51 tissue samples across 11 tumor types were tested.
  - The assay results were reproducible for tissue samples where there is less than 50% tumor included (Figure 4).

- Results for Testing Specific (Micro, Hemoglobin, Fat, Necrosis, Fibrosis, and TNFA Interferences)
  - Non-tumor lymphoid aggregates (NTLA) were defined as
    - Immunohistochemistry staining with a least one antibody not associated with an anti-tumor immune response (CD3, CD8, CD20, CD38, PD-1, PD-L1).
    - Normal lymph node tissue surrounding a tumor that has been verified.
  - More interfering TIS score range (Figure 5).
  - 51 tissue samples across 11 tumor types were tested.
  - The assay result was reproducible for tissue samples where there is less than 50% tumor included (Figure 4).

- Results for Testing Specific Interferents
  - The TIS assay requires pathologists to review H&E slides to identify tumor content which lab technicians provide in a database. The H&E review and tissue processing could cause variability in TIS score and biomarker classification.

- Deterministic TIS Score for each sample by TIS Score (K) = 2

- Table 1: Present concordance of TIS assay results between independent tissue replicates.

- Table 2: The mean score difference due to the inclusion of interferents and TIS.

- Table 3: The mean score difference due to the inclusion of interferents and TIS.

- Figure 1. FFPE Tissue Reproducibility Study Design

- Figure 2. TIS scores across 3 pathologists and 3 users for each of the TIS analyzed at 3 independent and core biopsy samples. Samples are colored by pathologist.

- Figure 3. Comparison of TIS score differences where adjacent tumor was removed vs. those where it was not removed.

- Figure 4. Comparison of the TIS score differences where adjacent interferent (NTLA) was included vs. those where it was not included.

- Figure 5. Comparison of the TIS score differences where NTSA was removed vs. those where NTSA was not removed, by each TIS assay and concordance.

Table 1. Number of samples, mean score bias and 95% confidence interval relative to the On Protocol assay.

<table>
<thead>
<tr>
<th>Interference</th>
<th>No Interference</th>
<th>Mean Score Bias</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td></td>
<td>0.00</td>
<td>-0.00,0.06</td>
<td>0.37 [0.23,0.52]</td>
</tr>
<tr>
<td>NTLA</td>
<td></td>
<td>0.03</td>
<td>-0.00,0.06</td>
<td>1.5</td>
</tr>
</tbody>
</table>

Conclusions

Our NanoString TIS assay is a reproducible and robust test, which profiles immune-related gene expression across multiple cancer types. The NanoString TIS assay is well suited for decentralized clinical testing with a turnaround time of 3 days or less (from sample receipt to test result).

- The Nanostring TIS assay score from tissue is reproducible across multiple pathologists and users with high biomarker classification concordance (+95% identical between individual tissue replicates and a total standard deviation representing less than 5% of the score range).
- Interference from genomic DNA, NTLA, and >50% adjacent non-tumor interferent can impact the TIS result and should be removed per the TIS protocol.
- TIS is robust against inclusion of micro, hemoglobin, fat, necrosis, tissue and fibrotic tissue interferents and provides accurate results.