Development of ddPCR blood-based diagnostic tests that simultaneously measure mRNA expression from immune and cancer cells.

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Abstract

Background: Therapeutic options for patients with non-small cell lung cancer (NSCLC) continue to expand with the advent of immunotherapies. Lack of tissue and drawbacks with available IHC tests has become highly relevant to clinical testing.

Methods: We focused on extending the utility of ddPCR-based testing for measurement of intracellular transcripts to multiplexed detection of gene expression. Specifically, we addressed maximizing the yield of quality circulating RNA for use in multiplexed droplet digital PCR (ddPCR) assays. Evaluation criteria included droplet counts for biomarkers of cancer and immunotherapy response.

Results: Cell lines expressing variable levels of CK19 and PD-L1 were used to establish assay sensitivity. In these experiments, the test system could detect the markers in the equivalent of a single cell. We evaluated specificity using RNA from these same cell lines, resting and activated lymphocytes, and monocytes. The assays demonstrated the expected specificities. Given the complexity of assessing PD-L1 in circulation because of its expression on immune cells, we established preliminary thresholds based on CK19 and PD-L1 expression levels in normal, healthy donors (n=35). Of the 79 NSCLC donors tested 49% expressed CK19 above the threshold, indicating they contained sufficient circulating RNA derived from tumor. These donor samples tested either positive for EGFR delE746-A750 or L858R (n=13), negative for EGFR (n=10) or positive for KRAS G12D (n=16). We observed only three donors that expressed PD-L1 at high levels. This was regardless of EGFR mutation status. In contrast, 30% of KRAS mutation-positive donors samples were above the pre-defined PD-L1 threshold (n=16 samples tested). These data are consistent with tissue-based studies that report trends higher of PD-L1 expression in KRAS positive NSCLC patients. Previous reports have also indicated that for EGFR wild-type patients, PD-L1 over expression may be considered a poor prognostic indicator of OS.

Conclusions: We have developed sensitive and specific methods that can be applied to gene expression studies in blood. We have shown feasibility of these methods by evaluating key immune and cancer-specific RNAs including for PD-L1. Evaluations are on-going with prospective sample collections to validate thresholds for this assay that may lead to its clinical utility.

The GeneStrat Test:

The GeneStrat test was developed to accurately detect specific gene fusions and detect mutations in NSCLC and melanoma. As a next generation technology, GeneStrat is capable of detecting the following fusions and mutations:

- EML4-ALK, ROS1, RET
- KRAS G12D
- EGFR L858R, EGFR del19 (E746-A750)
- ROS1 PDGFR-B
- ALK PDGFR-B
- RET PDGFR-B
- EGFR T790M

The GeneStrat test is designed for use in formalin-fixed, paraffin-embedded tissue samples. The test is currently available in the USA and Europe.

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Results (continued)

Figure 8. Correlation of Orthogonal Technologies to measure PD-L1 Expression. PD-L1 mRNA levels were measured in cancer cell lines (green), immune cells (blue), and normal healthy donor PEP (gray) by ddPCR and by QPCR. The data demonstrate the high concordance (r=0.957) between these two technologies.

Table 1: Expression of PD-L1 is Preferentially Associated with KRAS Mutations in NSCLC Donors.

<table>
<thead>
<tr>
<th>PD-L1 expression (High vs. Low)</th>
<th>PD-L1+/KRAS+ vs. PD-L1+/KRAS-</th>
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<td>PD-L1 Low vs. High</td>
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<td>Total PD-L1+/KRAS+ vs. PD-L1+KRAS-</td>
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PD-L1 Development Summary

- Pros:
  - Fits current Biodesix CLIA-certified lab workflows for RNA (meets 72 hour turn-around time)
  - Method is independent of CTC or other vesicle isolation preparation
  - Feasibility demonstrated with prospectively collected PEP and ddPCR assays

- Cons:
  - Dependendent on sample diversity for thresholding
  - Preference is to validate against approved antibody test
  - Technical Feasibility (including Sensitivity and Specificity):
    - Cell lines with variable expression levels of PD-L1 and CK19
    - Activated and resting immune cells
    - Healthy donors and prospectively collected NSCLC samples

- Next Step to is to Collaborate for Validation vs. IHC and other clinical factors

References

- Gregg IC and Rice NA. Journal for Immunotherapy of Cancer (2016); 4:48.