Analytical Validation of an Immune Response Assay for Classifying Solid Tumors

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Background:

Immune Report Card™ (IRC) is an assay designed to provide comprehensive factual information for clinicians to support the decision process around the use of immunotherapeutics with an emphasis on checkpoint inhibitors. IRC provides this information by analyzing the host immune response and multiple immunotherapy biomarkers using several testing modes and integrates the results into a single report supporting clinical decision making in the realm of immunotherapeutics.

Specifically, IRC uses five test modes:

1. RNA-seq to quantitatively measure transcript levels of 43 genes related to T-cell receptor signaling or other components of the immune cycle and 11 genes related to tumor infiltrating lymphocytes.
2. DNA-seq to estimate mutational burden.
3. MSI-PCR to assess microsatellite instability.
4. Fluorescent in situ hybridization (FISH) to detect copy number gain of PD-L1 and PD-L2.
5. Immunohistochemistry (IHC) to measure protein expression of PD-L1 in the context of an FDA-approved assay and also to provide the pattern of expression for this marker as well as for CD3 and CD8.

The studies described here were designed to validate the analytical performance of the RNA-seq and DNA-seq components of IRC within our CLIA laboratory.

Precision Medicine Using Immune Response Profiling

Methods:

Multiple sets of gold standard FFPE samples were used to validate IRC addressing the two NGS test modes that identify two distinct categories of molecular profiling: gene expression (GEX), and mutational burden (Mulb). Using previously characterized gold standard samples, we evaluated sequencing performance characteristics at the runs and sample levels. The validation addressed pre-analytical sample processing variables and analytical robustness from nucleic acid through NGS and data analysis.

GEX: RNA-seq using the Oncomine Immune Response Research Assay (Thermo Fisher)
Mulb: DNA-seq using the Comprehensive Cancer Panel (Thermo Fisher)
Platform: Ion Chef and SXXL sequencing system (Thermo Fisher)
Analysis: Torrent Suite (Thermo Fisher) and a custom pipeline

Accuracy was determined by comparing transcript and DNA level variants with those from established RT-PCR and NGS assays. Transcript stability in FFPE specimens was evaluated in serial sections from blocks with routine storage and compared to originating fresh frozen (FF) specimens and unstained slides.

Assay Workflow and Validation Process

Analytic Validation Results

A. RNA-seq Batch size

B. Multi-Correlation of IRC DNA-seq vs Whole Exome

C. RNA-seq: CD274 dilution series

D. Non Superimposed Correlation of IRC RNA-seq vs TaqMan

Figure 4: A. GEX with various batch sizes. B. Mulb comparing IRC DNA-seq with TCGA WES. C. Linearity of detection for CD274 (PD-L1) gene expression. D. GEX comparing IRC RNA-seq with TaqMan qPCR across 54 validated genes.

Reproducibility

A. A 32 yo male with Stage IV melanoma treated with ipilimumab + nivolumab that resulted in a partial response.

B. A 67 yo male with Stage IIIIB/IV NSCLC treated with nivolumab that resulted in a progressive disease.

While the immunotherapeutic approach to this patient was to target the PD-L1/PD-1 axis the expression of both of these was low to moderate.

Use Cases

Conclusion: RNA stability was demonstrated by high degree of correlation between matched FF and FFPE samples. Analytic precision was demonstrated by high correlation between RNA-seq and TaqMan results for genes evaluated. Immune signatures were maintained with variable RNA/DNA input amounts, altered tumor micro-environments and potential interferences demonstrating tolerance to typical sample types tested. Reproducibility results show little variation between runs, days and operators. The validation results indicate that IRC NGS, a complex RNA-seq and DNA-seq assay, is accurate, highly reproducible and is fully fit for clinical use in solid tumor immune profiling.

1: Research Use Only, NYS CLEP approval pending