Predicting Response to Immunotherapies Using Minimal Tissue: A Novel 27-Gene Immuno-Oncology Assay that Measures the Tumor Microenvironment

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INTRODUCTION

- Immunotherapies have revolutionized cancer treatment, however relatively few patients respond.
- We have previously described a 27-gene immuno-oncology (IO) algorithm which has demonstrated predictive capabilities for immune checkpoint inhibitors (ICIs) in NSCLC and TNBC superior to PD-L1 and TMB.
- The 27-gene IO algorithm assesses the tumor immune microenvironment (TIME) by combining aspects of immune response, surrounding stromal cell signaling, and tumor physiology – resulting in an Immuno-Oncology (IO) Score and Mesenchymal (M) Score.
- A real-time qPCR multiplexed assay has been developed to offer high-throughput, cost-efficient testing with the algorithm.
- In addition to addressing the unmet need of improved predictive response to immunotherapies, maintaining low tissue input requirements is necessary to increase the number of patients who would qualify for testing.

METHODS

- Archival NSCLC and TNBC FFPE specimens sectioned 5 µm for H&E staining and histopathological evaluation to determine total tissue area and tumor content.
- Tissue biopsies included; Resection, core needle biopsy (CNB), and fine needle aspirate (FNA).
- Multiple tissue inputs were prepared for 45 samples to measure the IO Score reproducibility.
- Sample QC metrics were determined empirically and verified with the CAP/CLIA validation.
  - Total RNA input of 50 ng required for cDNA synthesis (3.57 ng/µL is the minimum concentration required to achieve 50 ng of RNA).
  - A qPCR control Ct value of 32.77 is required to confirm sample amplification quality.

RESULTS

Table 1. RNA purification statistics. Resections, CNB, and FNA FFPE specimens were extracted at various tissue inputs. Greater than 95% of samples had an RNA concentration of ≥ 3.57 ng/µL at 2 mm² or greater tissue input.

FFPE Type	n	Min Tissue Input	Max Tissue Input	Min [RNA] ng/µL	Max [RNA] ng/µL	Ave [RNA] ng/µL
Resection (TNBC)	37	0.5 mm²	4.6 mm²	< 2.0	200	67.6
CNB (TNBC)	50	0.5 mm²	4.5 mm²	< 2.0	83.4	21.7
FNA (TNBC)	9	0.5 mm²	4.0 mm²	< 2.0	22.5	8.3
Resection (NSCLC)	98	0.7 mm²	10.6 mm²	< 2.0	343	43.9
CNB (NSCLC)	27	0.2 mm²	5.2 mm²	< 2.0	148	51.4
FNA (NSCLC)	29	0.5 mm²	4.0 mm²	< 2.0	128	18.5

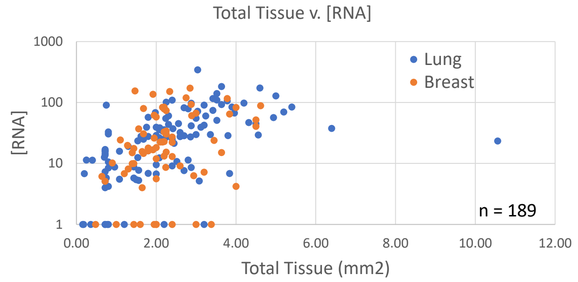

Figure 1. Scatter Plot of RNA concentration against total tissue input for Lung and Breast specimens.

Table 2. Replicate exactions from FFPE specimens with varying amounts of tissue input were performed. The total tissue input was tested in bins of 1mm² to determine minimal input required for successful PCR as defined by passing control gene threshold (>32.77). Greater than 98% of samples passed the control gene threshold QC metric with at least 2 mm² of tissue.

Tissue Input Ranges	% Pass Range qPCR	Range Pass qPCR	Range Total	Running Total
0.2 to 0.99 mm²	100%	27	27	98.8%
1 to 1.99 mm²	97%	35	36	98.6%
2 to 2.99 mm²	98%	60	61	99.1%
3 to 3.99 mm²	100%	31	31	100%
4+ mm²	100%	17	17	100%

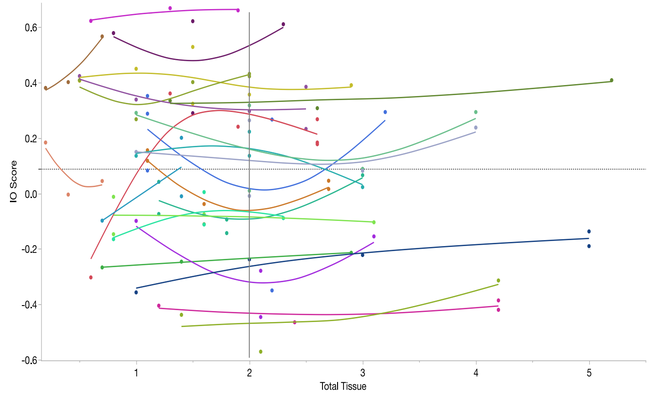

Figure 2. IO score by total tissue input (mm²) from multiple tissue inputs from the same sample as identified by line color. IO results are highly reproducible when 2 mm² or greater tissue input is present. We observed 90% reproducibility across the entire tissue input range using the IO Score threshold of 0.09.

Table 3. Replicate exactions from FFPE specimens with varying amounts of tissue input were performed. We calculated the difference form the Min and Max IO Score within each sample and averaged the difference stratifying by tissue input. We also calculated the coefficient of variance (CV) of the IO Score in order to assess the frequency of variation as a function of tissue input.

Tissue Input	Ave IO Score Difference	CV IO Score	n =
< 2 mm²	0.105	144%	24
≥ 2 mm²	0.112	18%	26

Table 4. Comparative Diagnostic test tissue input requirements.

Diagnostic Test	Minimum Tissue Input	Slide Requirement
27-gene IO	2 mm²	1-5 slides
PD-L1 (22C3) IHC†	‡6 mm²	3-5 slides
PD-L1 (28-8) IHC†	‡8 mm²	4-5 slides
PD-L1 (SP142) IHC†	‡6 mm²	3-5 slides
TMB*	250 mm²	10+ slides*

‡Average tissue mass from our study in 3 slides

RESULTS SUMMARY

- A total of 108/109 RNA isolations met the acceptance criteria of 3.57 ng/µL when using at least 2 mm² of tissue for both NSCLC and TNBC (Table 1).
- Tissue inputs ranged from 0.2 mm² to 10.6 mm² and RNA concentration varied from input size and biopsy type (Table 2, Figure 1).
- The 27-gene IO score was found to be highly reproducible between replicate extractions from 2 mm² of tissue or greater with > 90% reproducibility across the threshold (Figure 2).
- Stratifying samples by a 2 mm² tissue we observed a higher frequency of variation under 2 mm² tissue input (Table 3).

CONCLUSION

- At 2 mm² of tissue input, 95% of samples pass RNA QC metrics and 98% of samples pass qPCR QC metrics and reproducible IO Scores.

DISCUSSION

- PD-L1 IHC has provided unsatisfactory ICI prediction capabilities and TMB tissue requirements are too burdensome for many patient biopsies (Table 4).
- The 27-gene IO qPCR assay requires as few as 1 slide, results in greater than 95% QC success rate, and has demonstrated reliable predive capabilities to ICI therapies.

REFERENCES

† 3-5 slides recommended for PD-L1 IHC and may vary for each diagnostic test.
* 10 slides of 25 mm2 or greater tissue is requested for FoundationOne CDx TMB.

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