Transcriptomic Characterization of Immune Response within Diverse Tumor Environments using the NanoString® PanCancer IO 360™ Assay

Background

The efficacy of immune response in solid tumor settings is driven by many factors including the biology of the tumor, the immune system, and the microenvironment. The Tumor Inflammation Signature (TIS; RUO version) is an 18 gene signature that measures the presence of a preexisting immune response on the nCounter® platform and enriches for response to pembrolizumab (Ayers, JCI 2017). We have incorporated TIS into the PanCancer IO 360, a 770 gene expression panel containing 47 additional signatures of tumor-immune biology. To accompany this panel, we have created analysis software that associates the gene expression and signature scores with clinical annotations of the samples to characterize the immune system, tumor, and stroma within the tumor microenvironment to give insight into underlying biology of response to treatment, disease progression, survival, and other sample characteristics.

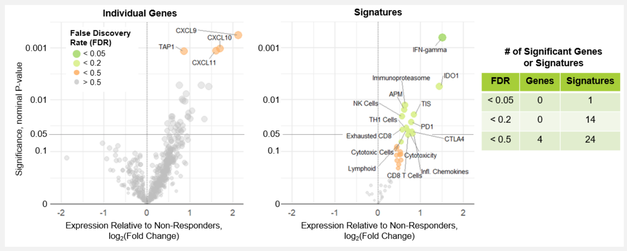
Methods

The PanCancer IO 360 assay relies on gene signatures to describe biological processes, measure the presence of 14 different immune cell populations, or report the expression of key therapeutic targets. Data from The Cancer Genome Atlas (TCGA) was used for signature training and development. Signatures are either single genes, weighted linear sums of multiple genes with coregulated expression, or algorithms to determine under-expression of genes in a coregulated pathway (Danaher JITC 2016; Danaher JITC 2019). The analysis software leverages differential expression analysis and Cox proportional hazard modeling to associate gene expression and signature scores with the clinical annotations.

Tumor (20%)	Microenvironment (20%)	Immune Response (60%)
16 Pathways and Processes		
34 Signatures Measuring Immune and Tumor Activity		
14 Cell Type Abundance Scores		
18-Gene Tumor Inflammation Signature (TIS): Measures peripherally suppressed adaptive immune response		

IO360 Signatures

IO360 Signatures Improve Statistical Power and Interpretation of Data



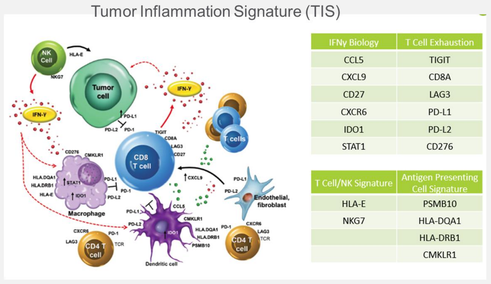
# of Significant Genes or Signatures		
FDR	Genes	Signatures
< 0.05	0	1
< 0.2	0	14
< 0.5	4	24

- Signatures help focus interpretation of results on the most relevant biology
- Reduce noise with signatures by smoothing technical noise across a pathway
- Reduce multiplicity of testing and decreases rate of false positives

IO360 Analysis of 48 Signatures

Tumor Immunogenicity	Tumor Sensitivity to Immune Attack	Inhibitory Tumor Mechanisms	Stromal Factors	Inhibitory Metabolism	Anti-Tumor Immune Activity	Inhibitory Immune Signaling		Immune Cell Population Abundance	
Antigen Processing Machinery	Apoptosis	IDO1 Gene Expression	Endothelial Cells	Glycolysis	Tumor Inflammation Signature (TIS)	CTLA4 Gene Expression	PD-L2 Gene Expression	B Cell Abundance	Mast Cell Abundance
Antigen Presenting Machinery Expression Loss	Tumor Proliferation	PD-L1 Gene Expression	Stromal Tissue Abundance	Hypoxia	Cytotoxicity	IL10 Gene Expression	TIGIT Gene Expression	CD45+ Cell Abundance	Neutrophil Abundance
Immunoproteasome	JAK-STAT Pathway Gene Expression Loss	B7-H3 Gene Expression			Interferon Gamma Signaling	Inflammatory Chemokines	ARG1 Gene Expression	CD8+ T Cell Abundance	NK CD56dim Cell Abundance
MAGE Genes Expression		TGF-Beta Gene Expression			Interferon Signaling Response	Myeloid-Derived Inflammatory Signaling	NOS2 Gene Expression	Cytotoxic Cell Abundance	Natural Killer Cell Abundance
Loss of Mismatch Repair Gene Expression					Lymphoid Compartment Activity	PD-1 Gene Expression		Dendritic Cell Abundance	T Cell Abundance
Hypermutation					MHC Class II Antigen Presentation			Exhausted CD8 Cell	TH1 Cell Abundance
MSI Predictor					Myeloid Compartment Activity			Macrophage Abundance	Treg Abundance

Includes the Tumor Inflammation Signature (TIS)



IFNγ Biology	T Cell Exhaustion
CCL5	TIGIT
CXCL9	CD8A
CD27	LAG3
CXCR6	PD-L1
IDO1	PD-L2
STAT1	CD276

T Cell/NK Signature	Antigen Presenting Cell Signature
HLA-E	PSMB10
NKG7	HLA-DQA1
	HLA-DRB1
	CMKLR1

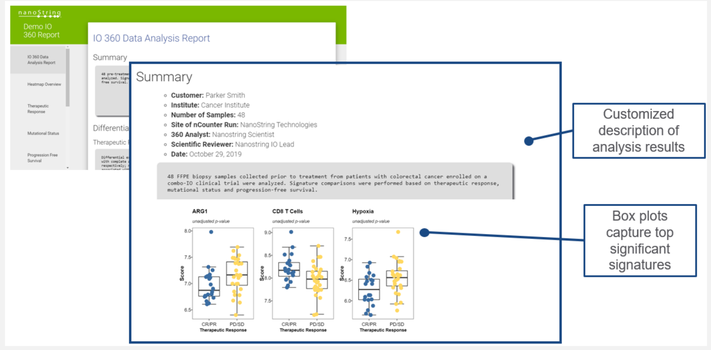
The Tumor Inflammation Signature (TIS) contains 18 genes that measure a peripherally suppressed immune response and distinguishes tumors as immune hot and cold (Ayers et al, JCI 2017)

IO360 Report

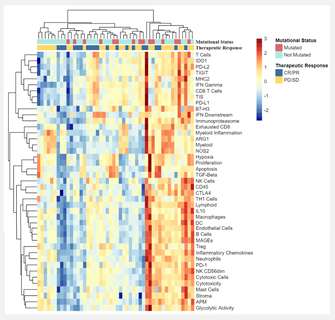
Get More Out of Your IO360 Data

- Get the most out of your IO360 panel data with the IO360 report
- Highly curated content developed in conjunction with leading experts in both academics and industry
- 48 signatures measuring biological variables crucial to the tumor-immune interaction
- Highly consultative nature of report delivery
- Ability to add signatures and genes of interest
- Ease of interpretation- statistics methods section, executive summary, annotated graphs
- Browsable report in HTML format with downloadable figures (no special software required)
- Ability to add up to 5 custom signatures or genes of interest
- Publication ready figures and statistical outputs

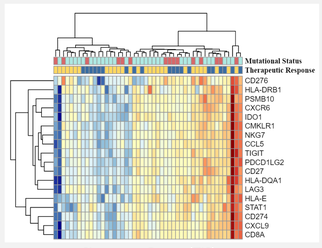
Executive Summary with Significant Findings



Heatmaps of Signatures and Genes



IO360 Signatures



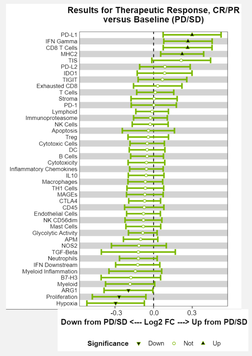
Signature Genes (TIS)

IO360 signatures and gene clustering analysis for all samples with a choice of annotations.

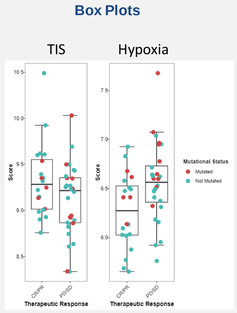
Heatmap types include:
- All signatures (single gene and metagene)
- Individual signatures with > 5 genes
- All genes in panel
- Spike-in genes can be treated as signature in report, including heatmap

Response Analysis

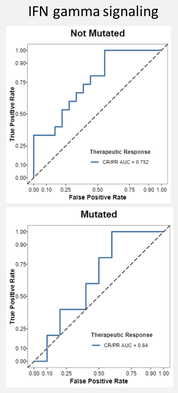
Forest Plot



Box Plots



Receiver Operating Curve (ROC)



Response can be assessed at the level of the population (forest plot), individual patients (box plots), Predictive performance of individual signatures can be evaluated with receiver operator curve (ROC) analysis.

Multiple Analysis Outputs and Comparisons

Analysis Outputs
- **Differential Expression**
  - Signature Level
  - Gene Level
- **Survival Analysis**
  - Signature Level
  - Gene Level
- **Analytical Plots**
  - Heatmaps
  - ROC Curves
  - Kaplan-Meier Curves
  - Wheel Plots
  - Scatter Plots
  - Box Plots
  - Forest Plots
  - Swimmer Plots
  - Volcano Plots

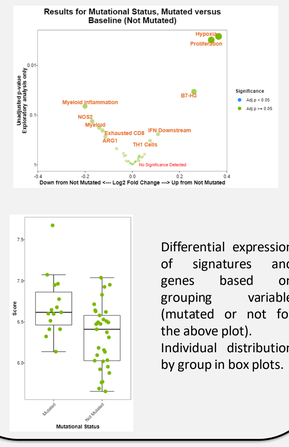
IO 360 Report Contents
- Summary
- Heatmap Overview
- Response Analysis*
- Grouping Analysis*
- Survival Analysis*
- Single Sample Analysis
- Loss Signature
- Quality Control Details
- Signature Descriptions
- Methods

* Report includes 3 tabs- any combination of Response, Grouping and Survival

Accessing the IO360 Report

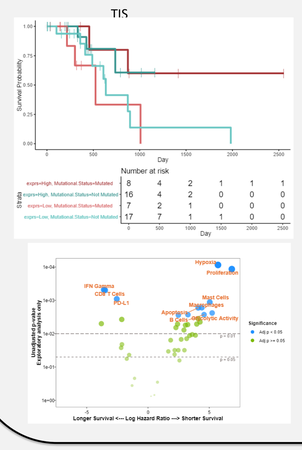
- IO360 report can be generated from any PanCancer IO360 data
  - Samples run in investigator lab, at CRO, or at NanoString
  - Annotated or unannotated data
  - Human IO360 data
  - Contact trp@nanostring.com or your account representative for more information

Grouping Analysis



Differential expression of signatures and genes based on grouping variable (mutated or not for the above plot). Individual distribution by group in box plots.

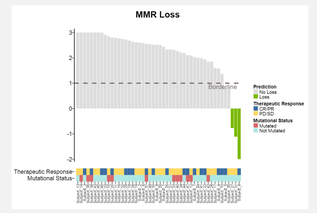
Survival Analysis

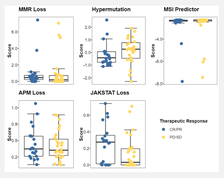


Kaplan Meir curves for high and low signatures and grouping variables (e.g. mutated or not mutated) with summary even and censored table.

Differential expression of signatures and genes based on hazard ratio. .

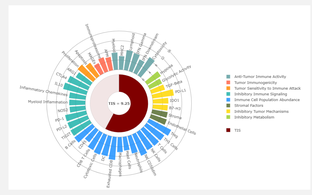
Gene Loss Signatures



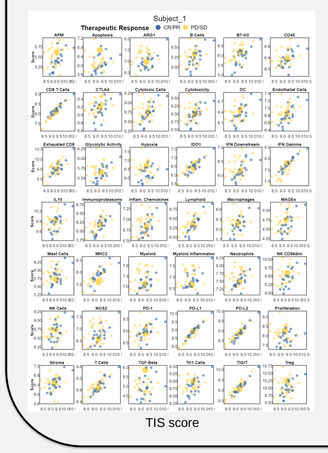


Loss signatures measure the expected loss of genes in a pathway (Antigen presenting machinery-APM, mismatch repair-MMR, JAK-STAT signaling or likelihood of hypermutation or microsatellite instability-MSI predictor.

Single Sample Analysis



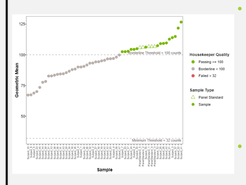
Wheel plot is the fingerprint of each sample showing individual scores by biological category (legend) and TIS in the center



Visualization of individual samples within the analyzed cohort for each IO360 signature.

TIS score

Quality Control Summary



- **QC Metrics Includes**
  - Housekeeping Gene QC
  - Imaging QC
  - Binding Density QC
  - Positive Control Linearity
  - Limit of Detection QC
- **Downloadable Tables.**
  - QC Flag Table
  - Sample Annotations

Conclusions

The PanCancer IO 360 assay is a tool for characterizing transcriptional patterns associated with tumor-immune interactions that can be applied across a wide range of cancer types. Gene signatures enable robust characterization of immune activity from small sample cohorts, and the report simplifies the interpretation of results. This combination enables researchers to have insight into clinically relevant biology that will ultimately lead to help drive the immune-oncology field

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