Abstract #205

Development of Gene Expression Signatures Characterizing the Tumor-Immune Interaction

NanoString Technologies 530 Fairview Ave North Seattle, WA 98109

Abstract

Background
The efficacy of anti-tumor immunity depends on diverse factors, including not just abundance of immune cell populations but also activities of those populations. Many of these processes are onerous or even impossible to assay, but all are reflected in a tumor's gene expression profile. Using a novel method, we develop gene expression signatures measuring a variety of biological processes underlying the tumor-immune interaction. These signatures fall into categories including antigen availability, structural barriers to immune infiltration, inhibitory signaling by both immune and tumor cells, inhibitory metabolism, pro-immune signaling, killing of tumor cells, tumor receptiveness to immune signaling, and tumor proliferation and death.

Methods
We develop a method to train signatures of biological processes by synthesizing biological knowledge and large gene expression datasets. For a given process, we use literature searches and expert knowledge to derive lists of candidate genes. We then evaluate the co-expression of these candidate genes in data from The Cancer Genome Atlas (TCGA), discarding genes whose co-expression patterns are incompatible with their measuring their putative biological process. This approach safeguards the interpretability of our signatures: we only report signatures whose genes show evidence for measuring the desired biology. Finally, we further exploit co-expression patterns to obtain optimal weights for each signature gene.

Results
We attempted to train signatures of over 30 biological processes involved in immune oncology. Of these, 17 candidate gene sets displayed sufficient evidence for measuring their putative biology. We show these signatures provide granular but intelligible descriptions of both immunotherapy datasets and single samples. We find they improve power in differential expression analyses and in training of predictors of drug response.

Conclusions
The signatures we derive convert gene expression data into measurements of biological processes central to immune oncology, and they improve statistical power and interpretation of results in immunotherapy studies. Our training procedure ensures these signatures measure their intended biology.

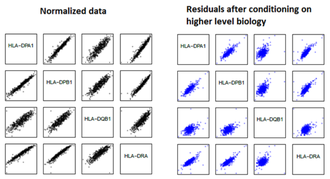
Development of Signatures

Training Method

Step 1: Use domain knowledge to identify genes participating in the process. (E.g. GZMA, PRF1, etc… for cytotoxicity.)
Step 2: Use domain knowledge identify potential confounding biological events (e.g. CD8 and NK cell abundance).
Step 3: Use TCGA to evaluate candidate gene co-expression and regress each candidate gene on scores for confounding biological events. Calculate residual of co-expression (i.e. measurement of co-regulation).
Step 4: Compare residual correlations to threshold to identify all genes that are tightly co-regulated for inclusion in signature. If fewer than half of genes are co-regulated, signature fails.
Step 5: Define weights for each gene so as to minimize the variance of the signature score from the actual levels of the process being measured.

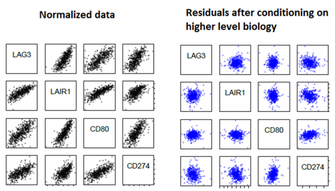
Example of a Successful Signature – MHC2 Antigen Presentation

Candidate genes: HLA-DPA1, HLA-DPB1, HLA-DQB1, HLA-DRA, etc…
Confounding Biological Processes: DC, B-cell, Macrophage, Lymphoid Cell, Myeloid Cells and Stromal Abundance; Tumor Cell Proliferation, data's first 3 PCs.
Result: Co-expression seen in unadjusted data remains after conditioning on confounding biology.



Example of a Failed Signature – Co-Inhibitory Signaling

Candidate genes: LAG3, LAIR1, CD80, CD274, etc…
Confounding Biological Events: T-cell, CD8 T cell, Lymphoid Cell, Myeloid Cells and Stromal Abundance; Tumor Cell Proliferation, data's first 3 PCs.
Result: Candidate genes fail to demonstrate show co-expression that is retained after condition on biological events
Other candidate signatures rejected for poor co-expression:
STAT1- regulated genes; Co-stimulatory signaling; Co-inhibitory signaling; Monocyte Abundance; MDSC Abundance; Myeloid DC Abundance; Eosinophil Abundance; WNT/β-catenin Signaling; Gluconeogenesis; Monocyte/MDSC Trafficking; BATF3 DC Trafficking; Autophagy; PTEN resistance; Fcγ-Receptor Expression



A Panel of Gene Expression Signatures for Interpreting the Tumor-Immune Interaction

Signatures and single genes detailing the tumor/immune interaction. Bold signatures were trained as part of this study. Other useful signatures and single genes were taken from the literature and analyzed alongside our new signatures. All the signatures in the table are included in the NanoString IO 360™ panel. The Tumor Inflammation Signature is an RUO version of the 18-gene signature developed by Ayers et al. that measures a peripherally suppressed adaptive immune system in the tumor. An IUO version of the TIS assay is available for retrospective or prospective use in clinical trials.

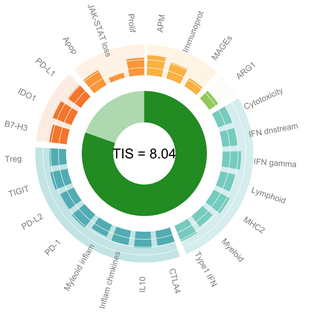
Tumor Immunogenicity	Tumor Sensitivity to Immune Attack	Inhibitory Tumor Mechanisms	Stromal Factors	Inhibitory Metabolism	Anti-Tumor Immune Activity	Inhibitory Immune Signaling		Immune Cell Population Abundance	
Antigen Processing Machinery	**Apoptosis**	IDO1 Gene Expression	**Endothelial Cells**	**Glycolysis**	Tumor Inflammation Signature	CTLA4 Gene Expression	PDL2 Gene Expression	B Cell Abundance	Mast Cell Abundance
Antigen Presenting Machinery Expression Loss	**Tumor Proliferation**	PD-L1 Gene Expression	**Stromal Abundance**	**Hypoxia**	**Cytotoxicity**	IL10 Gene Expression	TIGIT Gene Expression	CD45+ Cell Abundance	Neutrophil Abundance
Immunoproteasome	Loss of JAK-STAT pathway gene expression	B7-H3 Gene Expression			**Interferon Gamma Signaling**	**Inflammatory Chemokines**	ARG1 Gene Expression	CD8+ T Cell Abundance	NK CD56dim Cell Abundance
Loss of Mismatch Repair Gene Expression		TGF-Beta Gene Expression			**Interferon Signaling Response**	Myeloid-Derived Inflammatory Signaling	NOS2 Gene Expression	Cytotoxic Cell Abundance	Natural Killer Cell Abundance
MAGE Genes Expression					**Lymphoid Compartment Activity**	PD-1 Gene Expression		Dendritic Cell Abundance	T Cell Abundance
MSI-High Predictor					**MHC Class II Antigen Presentation**			Exhausted CD8	TH1 Cell Abundance
					Myeloid Compartment Activity			Macrophage Abundance	Treg Abundance

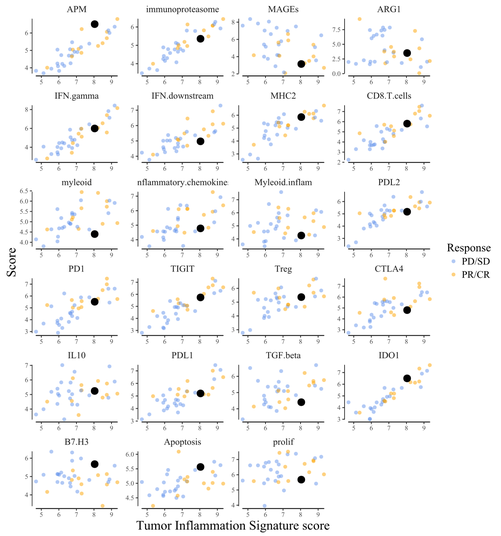
Single Sample Interpretation of IO Gene Signatures

When profiled together, these signatures allow comparison of a single sample to other samples within an experiment or to historical data. In this example, a melanoma tumor that failed to respond to anti-PD1 was profiled with the NanoString IO 360 panel. This tumor's failure to respond despite its high TIS may be attributable to its high B7-H3 expression.

Below: Individual sample signature scores.

Right: The same tumor is highlighted in plots comparing all signatures against the Tumor Inflammation Signature (TIS). Point color indicates drug response.



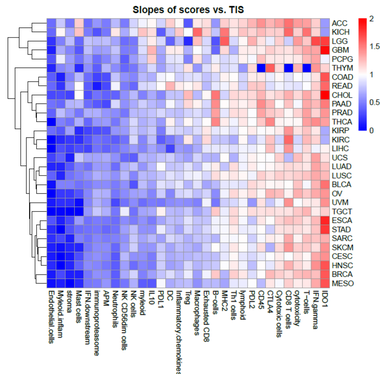


tumor foreignness
immune activity
immune inhibition - tumor intrinsic
immune inhibition - metabolism
immune inhibition - immune intrinsic
tumor sensitivity to immune attack

Pan-Cancer Study of IO Signatures: Analysis in TCGA

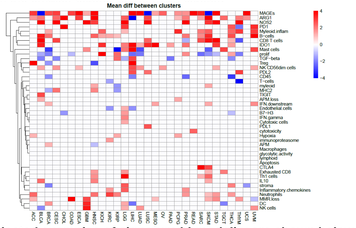
Co-expression of Signatures vs TIS to Characterize Gene Expression of Hot vs Cold Tumors

We use the Tumor Inflammation Signature (TIS) to measure the overall level of immune infiltration within a tumor. This heatmap shows the rate at which each signature increases as TIS increases. Signatures that are highly correlated with TIS (slope >1) are a prominent component of the immune infiltrate in immune hot tumors. In contrast, signatures that are not highly correlated with TIS tend to be enriched within in the immune infiltrate in immune cold tumors.

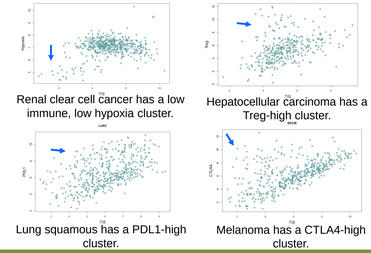


Search for clusters in signature scores

Variables with clusters or bimodality are often of additional biological interest or diagnostic relevance. To identify such patterns in our signatures, we regressed each signature against TIS and tested the residuals for bimodality. The below heatmap highlights statistically significant cases of bimodality, with color denoting the mean difference from the smaller cluster to the main cluster.



Selected examples of signature bi-modality are shown below.



Renal clear cell cancer has a low immune, low hypoxia cluster.

Hepatocellular carcinoma has a Treg-high cluster.

Lung squamous has a PDL1-high cluster.

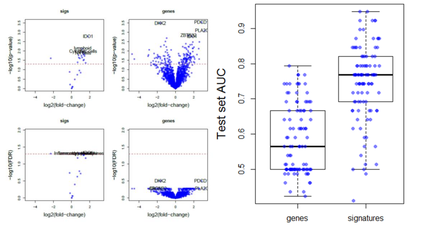
Melanoma has a CTLA4-high cluster.

Signatures Improve Statistical Power vs. Analysis of Individual Genes

Signatures simplify analyses and improve statistical power by reducing the number of variables being considered. This can allow stronger inferences to be drawn from a smaller sample size.

Right: volcano plots using p-values (top) and False Discovery Rates (bottom) from single genes (right) and signatures (left). After multiple-test correction, analysis of signatures yields more high-confidence conclusions.

Far Right: Anti-PD1 melanoma dataset was split into 50 random train-test sets. Predictors of response were trained in each training set and evaluated in the test sets. Predictors trained using signatures had better predictive power than those trained using single genes. (achieved higher area under the curve (AUC) in a receiver operator curve)



Conclusions

- The novel gene signatures described here provide a detailed description of tumor-immune interactions.
- The NanoString IO 360™ panel allows sample profiling with all the signatures using a single 770 gene expression panel and permits single-sample interpretation of these signatures.
- Use of gene signatures instead of single genes improves statistical power in machine learning and differential expression analyses.

Patrick Danaher, PhD, Sarah Warren, PhD, Xing Ren, MS, Afshin Mashadi-Hossein, MS, Mingdong Liu, MS, Joseph M Beechem, PhD, and Alessandra Cesano, MD PhD
NanoString Technologies, Seattle, WA USA
*Corresponding author pdanaher@nanostring.com