Top Immuno-oncology Publications

Tumor Inflammation Signature

IFN-γ-related mRNA profile predicts clinical response to PD-1 blockade
Ayers *et al., J Clin Invest*. 2017 Aug 1;127(8):2930-2940

The basis of the Tumor Inflammation Signature (TIS). The authors analyzed gene expression profiles using a Custom CodeSet consisting of 680 genes related to T cell biology, immune regulation, and cellular markers of tumor-infiltrating lymphocytes and tumor-associated macrophages. 50 ng of FFPE-derived RNA was used from baseline tumor samples of pembrolizumab-treated patients. The authors identified immune-related signatures correlating with clinical benefit using a learn-and-confirm paradigm based on data from different clinical studies of pembrolizumab, starting with a small pilot of 19 melanoma tumors and eventually defining a pan-tumor T cell-inflamed tumor inflammation signature (18 genes) in 220 samples across nine cancers.

Pan-cancer adaptive immune resistance as defined by the Tumor Inflammation Signature (TIS): results from The Cancer Genome Atlas (TCGA)
Danaher *et al., J Immunother Cancer.* 2018 Jun 22;6(1):63

TIS has been shown to enrich for tumors that respond to the anti-PD1 agent pembrolizumab and is a measure of pre-existing adaptive immunity that has been peripherally suppressed. This paper investigates how this immune phenotype distributes within and across tumor types, and how it correlates with other relevant variables such as mutational load, other gene expression signatures, and clinical outcomes in the absence of specific immune therapeutic intervention. The authors applied the Research Use Only (RUO) TIS algorithm to gene expression data from The Cancer Genome Atlas (TCGA) database of primary tumors

Pan-tumor genomic biomarkers for PD-1 checkpoint blockade-based immunotherapy
Cristescu *et al., Science.* 2018 Oct 12;362(6411)

Tumor mutational burden (TMB) and a T cell–inflamed gene expression profile (GEP) are emerging predictive biomarkers for pembrolizumab. Both PD-L1 and a T cell-inflamed GEP are inflammatory biomarkers indicative of a T cell–inflamed tumor microenvironment (TME), whereas TMB and microsatellite instability-high (MSI-H) are indirect measures of tumor antigenicity generated by somatic tumor mutations. However, the relationship between these two categories of biomarkers is not well characterized. The authors assessed the potential for

TMB and a T cell–inflamed GEP to jointly predict clinical response to pembrolizumab in >300 patient samples with advanced solid tumors and melanoma across 22 tumor types from four KEYNOTE clinical trials. RNA was isolated from 5-μm-thick FFPE sections of tumor tissue. 50 ng of total RNA was used with a Custom CodeSet. The analysis showed that TMB and inflammatory biomarkers (a T cell–inflamed GEP and PD-L1 expression) can jointly stratify human cancers into groups with different clinical responses to pembrolizumab monotherapy and identify patterns of underlying, targetable biology related to these groups. TMB and inflammatory biomarkers independently predict response and may capture distinct features of neoantigenicity and T cell activation, respectively.

Immuno-oncology

Microenvironmental Immune Cell Signatures Dictate Clinical Outcomes for PTCL-NOS
Sugio *et al., Blood Adv.* 2018 Sep 11;2(17):2242-2252

Peripheral T cell lymphoma (PTCL) not otherwise specified (PTCL-NOS) is among the most common subtype of PTCL. Prognosis of PTCL-NOS patients is dismal: the 5-year survival rate is as low as 30% due to lack of clinically meaningful disease-stratification models and effective therapies. Given PTCL-NOS heterogeneity, identifying molecularly and/or clinically distinct subgroups is necessary to develop novel therapeutic strategies. The authors goal was to establish a novel disease-stratification model based on quantitative measurement of gene expression signatures derived from both cancerous T cells and the microenvironment.

FFPE tumor tissues from 68 PTCL-NOS samples were analyzed. RNA was extracted from FFPE samples using the RNeasy FFPE extraction kit after treatment with deparaffinization solution. Gene expression levels were assessed using 300 ng of total RNA and run with the PanCancer Immune Profiling Panel.

The group analyzed levels of transcripts derived from tumors and microenvironment immune cells. Because standard mRNA expression analysis, such as microarray and RNA sequencing, is not sensitive enough to reliably measure transcripts expressed at low levels in microenvironment cells, they used the nCounter® system, which enables accurate quantification of low abundance, highly fragmented transcripts obtained from FFPE samples.

The authors wrote, "Interrogating tumor-infiltrating immune cells, which comprise a small population in tumors, quantitatively is

technically challenging due in part to detection limits of microarray and RNA-sequencing methods. The nCounter system enabled us to quantitatively measure transcript levels in both tumor and microenvironmental cells without polymerase chain reaction (PCR) amplification and to detect highly fragmented RNAs in FFPE samples."

Comparison of Gene Expression and Flow Cytometry for Immune Profiling in Chronic Lymphocytic Leukemia
Sharpe *et al., J Immunol Methods.* 2018 Dec;463:97-104

Flow cytometry is the gold standard method for profiling peripheral blood immune cells; however, the requirement for viable cells can limit its applicability, especially in studies of retrospective clinical cohorts.

Immune profiling of longitudinal samples allows for analysis of the *in vivo* changes to immune populations over time. The authors' objective was to evaluate two methods of quantifying peripheral blood immune cell populations, flow cytometry and gene expression analysis, in the context of Chronic Lymphocytic Leukemia (CLL).

The group simultaneously analyzed peripheral blood mononuclear cell (PBMC) samples using flow cytometry and the PanCancer Immune Profiling Panel. Of the possible 14 immune populations reportable by the nCounter Advanced Analysis software, 12 immune populations were determined to be significantly supported by the expression data (p <0.1). Of these, the two flow cytometry panels identified seven populations: B cells, total T cells, CD8 T cells, CD4 T cells, total NK cells, CD56dim NK cells and Monocytes. The group observed a strong positive correlation between the abundance score and the immune population frequency calculated using flow cytometry for all immune populations analyzed.

The group demonstrated that the research-use-only PanCancer Immune Profiling Panel and accompanying gene expression analysis software could provide immune cell abundance quantification comparable to flow cytometry in samples from CLL patient samples with widely divergent immune profiles. Gene expression analysis and flow cytometry resulted in significantly correlated immune abundance measurements for all cell types assessed, including NK and T cell subpopulations.

While flow cytometry requires design and optimization depending on the cell populations of interest, the PCIP and associated cell profiling advanced analysis feature is an off-the-shelf product

which can be used in a variety of experiments where the immune response is of interest. Furthermore, the nCounter platform only requires 25–100 ng of total RNA, which can be extracted from far fewer cells than the amount needed for flow cytometry analysis.

Evolution of Metastases in Space and Time Under Immune Selection
Angelova *et al., Cell.* 2018 Oct 18;175(3):751-765.e16

How do tumors evolve and metastasize? Previously, the Galon lab made efforts to elucidate the role of the tumor microenvironment on the metastatic potential of tumor cells but were hindered by the vast diversity of infiltrating immune cells. To date, very few studies have addressed the role of the immune system in metastatic heterogeneity. This publication examines, in a longitudinal dataset of colorectal cancer, how the immune microenvironment molds tumor evolution and metastasis to different organs.

The PanCancer Immune Profiling Panel was used on 27 samples to measure relative expression levels of immune genes within the tumor microenvironment. Total RNA (300 ng) was assayed from FFPE samples. An additional Custom CodeSet consisting of 48 immune specific targets was also used. Interestingly, immune gene expression using the PCIP correlated very well with immunoediting but not with mutational load. Characterization of immune-privileged metastases revealed tumor-intrinsic and tumor-extrinsic mechanisms of escape. The group proposed a parallel selection model of metastatic progression where branched evolution could be traced back to immune-escaping clones.

Tumor microenvironment remodeling by intratumoral oncolytic vaccinia virus enhances the efficacy of immune checkpoint blockade
Chon *et al., Clinical Cancer Research* 25(5):2019

There are many reviews on the subject, but certainly to date oncolytic viruses have shown great promise in cancer with the possible advantages of stronger efficacy compared to conventional therapy due to higher tumor selectivity and less toxicity. They preferentially and selectively propagate in cancer cells, consequently destroying tumor tissue mainly via cell lysis, while leaving non-cancerous tissues unharmed. Several wild-type and genetically engineered vaccinia virus (VACV) strains have been tested with promising results.

Cancer immunotherapy is a potent treatment modality, but its clinical benefit depends on the tumor's immune profile. The

group employed mJX-594 (JX), a targeted and GM-CSF–armed oncolytic vaccinia virus, as a strategy to remodel the TME and subsequently increase sensitivity to anti-PD-1 and/or anti-CTLA-4 immunotherapy. Using a variety of methods including flow and the use of the PanCancer Immune Profiling Panel, the group extracted and profiled RNA from mouse models. The JX vaccinia virus was intratumorally injected into implanted Renca kidney tumors or MMTV-PyMT transgenic mouse breast cancers with or without anti-PD-1 and/or anti-CTLA-4.

Results using NanoString indicated that JX elicits long-term immune activation through dynamic changes in the TME to remodel non-inflamed tumors into T cell–inflamed tumors that can respond to immune checkpoint inhibition.

MEK inhibition enhances oncolytic virus immunotherapy through increased tumor cell killing and T cell activation
Bomareddy *et al., Sci Transl Med.* 2018 Dec 12;10(471)

To date, approximately 40 to 50% of cutaneous melanomas harbor mutations in BRAF, which serve as oncogenic drivers of the MAPK pathway promoting tumor progression. Small-molecule inhibitors of BRAF and MAPK kinase (MEK) in treatment-naive melanoma whose tumors harbor V600E or V600K BRAF mutations contribute to significant improvements in relapse-free and overall survival.

Preclinical studies have suggested improvements in antitumor activity between oncolytic viruses and MEK inhibition in a murine breast cancer model. The combination of MEK inhibition and oncolytic viruses has not been tested in melanoma and has not yet entered clinical trials.

The group hypothesized that MEK inhibition would improve oncolytic virus responses in melanoma and sought to test this with currently approved agents in melanoma. In their mouse model, C57BL/6J mice were implanted subcutaneously in the right flank with 3×10^5 D4M3A melanoma cells and treated with the oncolytic virus mT-VEC (1×10^6 PFU) intratumorally for three doses on days 15, 19, and 22 and/or the MEK inhibitor trametinib (0.5 mg/kg) orally once daily on days 15 to 19. Tumors were harvested on day 24, total RNA was isolated, and gene expression analysis was performed using the PanCancer Immune Profiling Panel.

The group evaluated the combination of MAPK inhibition and T-VEC in murine and human melanoma cell lines and found a synergistic effect between T-VEC and MEK inhibition regardless of BRAF mutation status. They also confirmed that therapeutic responses could be further improved by addition of anti–PD-1 therapy. In their studies, they did not observe overt signs of toxicity in mice, supporting an improved therapeutic window (although clinical confirmation is needed).

For more information, please visit nanostring.com