WHITEPAPER

Multiplexed Cancer Immune Response Analysis

nCounter® PanCancer Immune Profiling Panel for Gene Expression

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Introduction

The ability of mutated cells to give rise to pathological cancer relies upon the capability of these cells to evade immune recognition, suppress immune activity, and persist in a chronically inflamed environment[1,2]. Tremendous growth in the understanding of these complex processes is beginning to generate breakthroughs in the treatment of cancer, and "Cancer Immunotherapy" was named the "Breakthrough of the Year" by Science in 2013[3]. However, our understanding of the immune response to cancer is far from complete. There is a need for tools that enable researchers to explore the tumor microenvironment in new and different ways. This report describes a novel gene expression panel that enables researchers to create profiles of the human immune response in all cancer types and shows how this panel has the potential to accelerate the development of drugs, therapies, and predictive biomarker signatures for response to immunotherapeutic treatments.

The nCounter® PanCancer Immune Profiling Panel is a highly multiplexed gene expression panel designed to quantify 770 genes that fall into four functional categories (**Figure 1**):

- **Immune cell profiling,** such as those in a PBMC population or infiltrating into a tumor.
- **Assessing immunological function** and response to immunotherapy, such as immune checkpoint regulation.
- **Identifying tumor-specific antigens**, such as cancer-testis (CT) antigens.
- **Housekeeping genes** that facilitate sample-to-sample normalization.

The nCounter PanCancer Immune Profiling Panel is fully compatible with clinically relevant sample types such as fresh-frozen (FF) tissue, formalin-fixed, paraffin-embedded (FFPE) tumor sections, isolated immune cell populations such as PBMCs, and cell lysates. The panel may be used in conjunction with nCounter® Panel-Plus for additional flexibility in experimental design.

Identifying Immune Cell Types in Cancer

Many immune cell types (**Table 1**) are found in the tumor microenvironment and interact with a tumor, creating a complex milieu that affects the growth and evolution of cancerous cells through promoting angiogenesis, inducing immune tolerance, and immunoediting[4-6]. Identifying and observing discrete cellular populations within samples has been a focus of immunological study for decades. Histopathological and flow cytometric analyses have provided ample evidence that variable numbers of infiltrating immune cells are found within the tumor microenvironment[7,8].

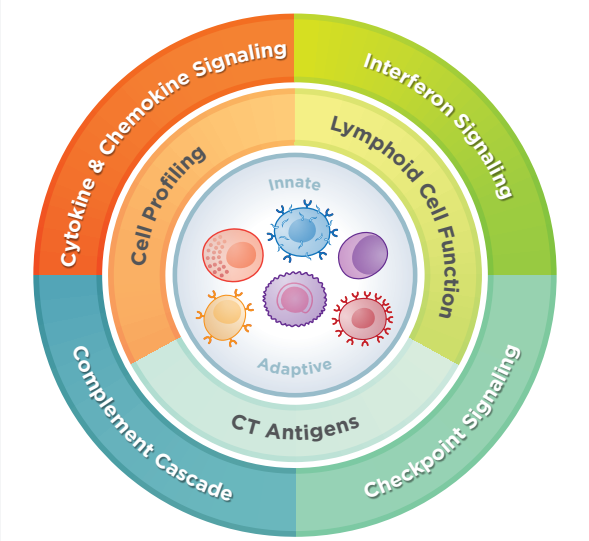

Figure 1: Distribution of genes included in the PanCancer Immune Profiling Panel, including genes for profiling immune cells, the innate and adaptive immune response, CT antigens, lymphoid cell function, cytokine and chemokine signaling, interferon signaling, checkpoint signaling, and complement cascade.

The classification and enumeration of immune cells within tumor samples and in the periphery has been shown to be a significant and powerful predictor of patient survival[9,10] and has led to efforts such as Immunoscore, an approach for classifying cancer pathologies[11,12]. By including markers demonstrated to specifically identify major immune cell populations within cancer samples[13] (**Table 1**), the PanCancer Immune Profiling Panel can efficiently define both the immunological activity of these samples as well as identify changes in immune cell populations in response to external stimuli such as immunotherapeutic adjuvants.

NanoString has included 109 genes that are associated with 24 immune cell types and populations. Genes were chosen after careful review of literature that included studies examining expression in purified cell populations[13]. For some cell populations, such as T cells, both pan T cell and lineage-specific markers are included, *e.g.*, observation of CD3E expression in a tumor sample could indicate infiltration of T cells while observation of FOXP3 in the same sample would specifically identify the presence of regulatory T cells, enabling the detection of rare cell populations.

IMMUNE CELL TYPES

	Cell Type			Description	Panel Genes
Adaptive Immune Response	B Cells			Perform several roles, including generating and presenting antibodies, cytokine production, and lymphoid tissue organization.	BLK, CD19, CR2 (CD21), HLA-DOB, MS4A1 (CD20), TNFRSF17 (CD269)
	T Cells			Play a central role in immunity and distinguished from other lymphocytes (*e.g.*, B cells) by the presence of a T cell receptor (TCR) on the cell surface.	CD2, CD3E, CD3G, CD6
		Helper T Cells		A subset of CD3+CD4+ effector T cells that secrete cytokines with different activities.	ANP32B (APRIL), BATF, NUP107, CD28 (CD28), ICOS (CD278)
			T_H1	Produce IL-2 and IFNγ and promote cellular immunity by acting on CD8+ cytotoxic T cells, NK cells and macrophages.	CD38, CSF2 (GM-CSF), IFNG, IL12RB2, LTA, CTLA4 (CD152), TXB21, STAT4
			T_H2	Produce IL-4, IL-5 and IL-13 and promote humoral immunity by acting on B cells.	CXCR6 (CD186), GATA3, IL26, LAIR2 (CD306), PMCH, SMAD2, STAT6
			T_H17	Produce IL-17A, IL-17F, IL-21 and IL-22 and promote anti-microbial inflammation.	IL17A, IL17RA (CD217), RORC
			T_{FH}	Trigger the formation and maintenance of germinal centers through the expression of CD40L and the secretion of IL-21 and IL-4, thereby playing a critical role in mediating the selection and survival of B cells.	CXCL13, MAF, PDCD1 (CD279), BCL6
		Regulatory T Cells (T_{reg})		CD3+CD4+ T cells that inhibit effector B and T cells and play a central role in suppression of autoimmune responses.	FOXP3
		Memory T Cells	T_{cm} (central memory)	Educated T cells that rapidly respond to antigen. Central memory T cells express L-selectin and CCR7; they secrete IL-2, but not IFNγ or IL-4.	ATM, DOCK9, NEFL, REPS1, USP9Y
			T_{em} (effector memory)	Educated T cells that rapidly respond to antigen. Effector memory T cells do not express L-selectin or CCR7; they secrete effector cytokines like IFNγ and IL-4.	AKT3, CCR2 (CD192), EWSR1 (EWS), LTK, NFATC4
Innate Immune Response	Cytotoxic Cells	Cytotoxic (CD8) T Cells		Effector T cells with cytotoxic granules that interact with target cells expressing cognate antigen and promote apoptosis of target cells.	CD8A (CD8), CD8B (CD8B), FLT3LG, GZMM (MET1), PRF1
		Gamma Delta T Cells (Tγδ)		Express surface antigen recognition complex type 2 and represent a small percentage of the peripheral T cell population. Functions span innate and adaptive immune responses, including direct cytolysis and establishment of memory phenotypes.	CD160, FEZ1, TARP (TCRG)
		Natural Killer (NK) Cells		Provide a rapid cytotoxic response to virally infected cells and tumors. These cells also play a role in the adaptive immune response by readily adjusting to the immediate environment and formulating antigen-specific immunological memory.	BCL2, FUT5, NCR1 (CD335), ZNF205
			$CD56_{bright}$	Constitute the majority of NK cells in secondary lymphoid tissues. Abundant cytokine producers and weakly cytotoxic before activation.	FOXJ1, MPPED1, PLA2G6, RRAD
			$CD56_{dim}$	Constitute the majority of NK cells in the periphery and are more cytotoxic than $CD56_{bright}$ cells.	GTF3C1, GZMB, IL21R (CD360)
	Dendritic Cells	Conventional (Myeloid) Dendritic Cells (DC)		Cells that process antigen material and present it on the cell surface to T cells, thereby acting as messengers between the innate and adaptive immune systems.	CCL13, CCL17, CCL22 (MDC), CD209 (CD209), HSD11B1
			iDC (immature)	Play a critical role in initiating tumor immunity. Tumor cells can exploit the functional roles of iDCs for tumor progression via release of soluble factors such as VEGF.	CD1A, CD1B, CD1E, F13A1, SYT17
			aDC (activated)	Promote the induction of the adaptive immune response by presenting captured antigen to naive T cells.	CCL1, EBI3, IDO1 (INDO), LAMP3 (CD208), OAS3
		Plasmacytoid Dendritic Cells (pDC)		Similar in appearance to plasma cells and share many characteristics with myeloid dendritic cells. These cells can produce high levels of IFNα.	IL3RA (CD123)
	Macrophages			Scavengers of dead or dying cells and cellular debris. Macrophages have roles in innate immunity by secreting pro-inflammatory and anti-inflammatory cytokines.	APOE, CCL7 (FIC), CD68, CHIT1, CXCL5, MARCO, MSR1 (CD204)
	Granulocytes	Mast Cells		Granulocytes that can influence tumor cell proliferation and invasion and promote organization of the tumor microenvironment by modulating the immune response.	CMA1, CTSG, KIT (CD117), MS4A2, PRG2, TPSAB1
		Neutrophils		Phagocytic granulocytes that act as first-responders and migrate towards a site of inflammation. Typically a hallmark of acute inflammation.	CSF3R (CD114), FPR2, MME (CD10)
		Eosinophils		White blood cells responsible for combating multicellular parasites and some types of infections.	CCR3 (CD193), IL5RA (CD125), PTGDR2 (CD294; GPR44), SMPD3, THBS1

Table 1: Immune cell types and populations involved in the response to cancer and corresponding population-specific genes included in the PanCancer Immune Profiling Panel.

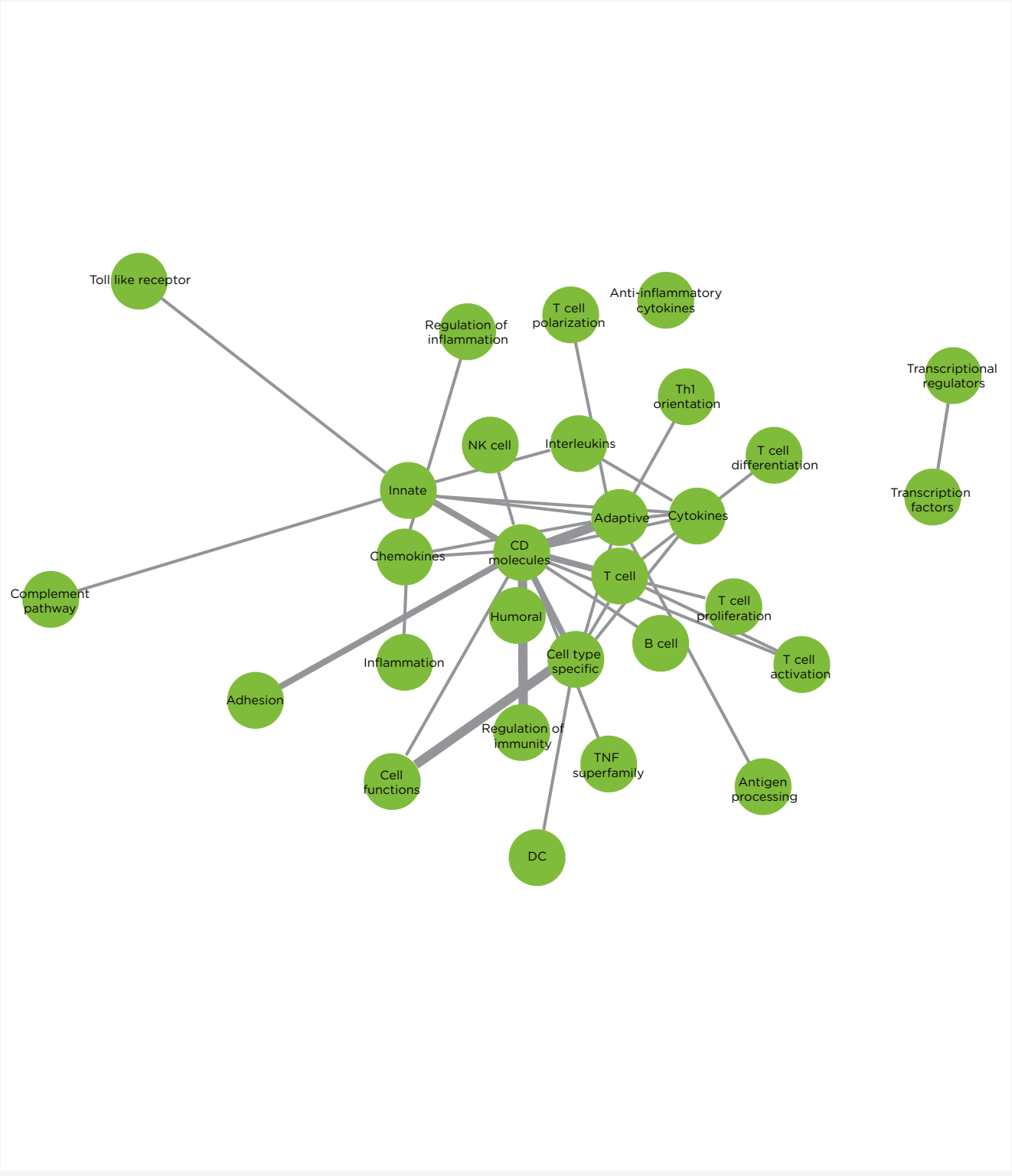

Figure 2: Network map of biological process annotations for Immune Response genes in the PanCancer Immune Profiling Panel. Each biological process is represented by a node, lines between nodes denote shared classifications for a gene, with the thickness of the line representative of the total number of genes with a given shared classification.

Cell Type	Description	Panel Genes
B Cells	B cells are the primary mediators of the humoral immune response, bearing antigen-specific B cell receptors and producing antibodies that can enable the immune system to respond to a broad variety of antigens. B cells can also function as MHC class II antigen presenting cells to stimulate T cell immunity.	BLK, CD19, CD20, TNFRSF17
T Cells	T-cells mediate cell-based immunity by recognizing primarily peptide antigens displayed on MHC class I or class II and either producing cytokines or directly killing the presenting cell.	CD3D, CD3E, CD3G, CD6, SH2D1A
TH1	CD4+ T cell subset that produces IL2 and Interferon-gamma to promote cellular immunity by acting on CD8+ T Cells, NK Cells and Macrophages	TBX21
Regulatory T cells (Treg)	CD4+ T Cells that supress effector B and T Cells and play a central role in suppresion of the immune response and tolerance to self-antigens	FOXP3
CD45	CD45 is a common marker of all leukocytes, including B and T cells	CD45
CD8+ T Cells	A subset of T cells that are capable of binding cognate-antigen expressing cells via class I MHC and directly lysing them via perforin and granzymes.	CD8A, CD8B
Exhausted CD8+ T Cells	T-cells overstimulated by antigen can develop an "exhausted" phenotype, in which they are no longer effective in targeting antigen-bearing cells.	CD244, EOMES, CD223
Cytotoxic Cells	These markers measure all cells capable of cytotoxic activity, which can include T, NKT, and NK-cells.	CTSW, GNLY, GZMA, GZMB, GZMH, CD161, CD94, KLRK1, PRF1
Dendritic Cells	Professional antigen presenting cells that internalize, process, and present antigens to lymphocytes via MHC class I and class II along with costimulatory signals to initiate cellular immune responses.	CCL13, CD209, HSD11B1
Macrophages	Pluripotent cells with critical roles in initiating innate and adaptive immune responses, phagocytosing abnormal cells, and regulating wound healing and tissue repair.	CD163, CD68, CD84
Mast Cells	Mast cells release histamine containing granules and other signals in order to promote inflammation and regulate allergic responses.	MS4A2, TPSAB1
Neutrophils	Neutrophils are highly abundant cells that respond early to sites of infection or inflammation, phagocytose cellular debris, and promote downstream immunity.	CSF3R, CD16, S100A12
Natural Killer (NK) Cells	Cytotoxic cells of the innate immune system that are a significant source of interferon-gamma and are capable of directly killing targeted cells via detection of a loss in MHC surface expression	NKP46, XCL2
NK CD56dim cells	The amount of CD56 present on an NK cell is indicative of its age and differentiation state; CD56 dim cells are mature NK cells, more commonly found in peripheral blood than secondary lymphoid tissues, and have the greatest cytolytic activity.	IL21R, KIR2DL3, KIR3DL1, KIR3DL2

Table 2: Immune cell types and selected human genes included in the Immune Cell Profiling feature embedded within the PanCancer Immune Profiling Panel which allows for calculating the relative abundance of 14 different immune cell types.

Gene Name	Additional Aliases
BAGE	CT2.1, BAGE1
CT45A1	CT45-1, CT45.1
CTAG1B	CTAG, CTAG1, NY-ESO-1, LAGE2B, LAGE2A, ESO1, CT6.1
CTAGE1	cTAGE-1, cTAGE-2, CTAGE, CT21.1, CT21.2
CTCFL	dJ579F20.2, BORIS, CT27
DDX43	HAGE, DKFZp434H2114, CT13
GAGE1	CT4.1
MAGEA1	MAGE1, MGC9326, CT1.1
MAGEA12	MAGE12, CT1.12
MAGEA3	MAGE3, HYPD, HIP8, MGC14613, CT1.3
MAGEA4	MAGE4, MAGE4A, MAGE4B, MAGE-41, MAGE-X2, MGC21336, CT1.4
MAGEB2	DAM6, MAGE-XP-2, MGC26438, CT3.2
MAGEC1	MAGE-C1, CT7, MGC39366, CT7.1
MAGEC2	MAGEE1, CT10, MAGE-C2
PASD1	CT63
PBK	TOPK, FLJ14385, Nori-3, SPK, CT84
PRAME	MAPE, CT130
PRM1	CT94.1
ROPN1	ODF6, ropporin, ROPN1A, CT91
SEMG1	SEMG, CT103
SPA17	SP17, CT22
SPACA3	ALLP17, SLLP1, LYC3, LYZL3, CT54
SPANXB1	CT11.2
SPO11	CT35, SPATA43, TOPVIA
SSX1	CT5.1
SSX4	CT5.4
SYCP1	HOM-TES-14, SCP1, CT8
TMEFF2	TENB2, HPP1, TR, TPEF, CT120.2
TPTE	PTEN2, CT44
TTK	MPS1, MPS1L1, CT96, MPH1

Table 3: CT Antigens profiled by the PanCancer Immune Profiling Panel.

The PanCancer Immune Profiling Panel also includes a unique cell profiling feature that uses gene expression data from 46 human genes to quantify the relative abundance of 14 immune cell types: B and T cells, Th1, Tregs, CD45 and CD8+ T cells, exhausted CD8+ T cells, cytotoxic and dendritic cells, macrophages, mast cells, neutrophils, natural killer (NK) cells, and NK CD56dim cells. (**Table 2**).[14]

Measuring Gene Expression Associated with Immunological Function

In their seminal review, Chen and Mellman describe a series of steps that must be initiated and fostered for an anticancer immune response to effectively kill cancer cells, dubbing these steps the Cancer Immunity Cycle[15]. This cycle begins with the release of antigen by cancer cells. Released antigens are processed and then presented, which results in priming and activation of the adaptive immune response. Once activated, T cells traffic to the tumor, infiltrate, and recognize tumor cells, ultimately leading to their programmed destruction. When cancer cells are killed by invading T cells, additional antigen is released into the periphery and starts the cycle anew. Regulation of these complex immunological processes involves hundreds of genes, many of which function in multiple biological processes. All genes included in the panel were chosen after a careful review of scientific literature and vetting with members of the cancer immunology research community. Each gene is annotated with major immunological function/process information from the Gene Ontology Consortium (www.geneontology.org)[16, 17].

Over 20 major biological processes are annotated (**Figure 2**), including inhibitory receptors that prevent uncontrolled T cell activation, *i.e.*, checkpoint regulation, which explains the failure of immune protection in many patients[18, 19].

A full list of genes found in the PanCancer Immune Profiling Panel and their annotated functions is documented and available at www.nanostring.com. In addition to functional annotations, the HUGO Gene Nomenclature Committee (www.genenames.org) name and commonly used aliases are described

Identifying Tumor-Specific Antigens

Cancer is characterized by the accumulation of genetic alterations and the loss of normal cellular regulatory processes[20, 21]. These events have long been known to result in the expression of cancer-specific antigens, which distinguish these cells from their normal counterparts[22]. These unique cancer-antigens are processed by antigen presenting cells, *e.g.*, dendritic cells, and are presented as peptides bound to major histocompatibility class I (MHCI) and II (MHCII) molecules to stimulate tumor antigen-specific T cells.

Among all tumor-specific antigens, cancer testis (CT) antigens —their expression is normally restricted to adult testicular germ cells[23] — have been found to elicit spontaneous humoral and cell-mediated immune responses in cancer patients. This makes CT antigens an attractive target for eliciting a specific immune response by immunotherapy[24,25]. The PanCancer Immune Profiling Panel contains probes for 30 of the most frequently studied and currently clinically relevant CT antigens (**Table 3**).

Measuring Housekeeping Gene Activities for Sample-to-Sample Normalization Comparison

The Cancer Genome Atlas (TCGA) expression data were examined across twelve tumor types and used to identify a subset of genes with consistent patterns of expression between cancers. These genes were selected as candidate housekeepers and are included in the PanCancer Immune Profiling Panel to aid data normalization. To demonstrate the impact of normalization, six healthy donor PBMC samples were profiled and gene content was normalized using the geometric mean of all 40 housekeepers. Normalization resulted in reduced expression variance across a range of expression levels with the highest expressing genes showing the greatest reduction (**Figure 3**).

In any particular dataset, some housekeepers may not be stably expressed and optimal normalization may be achieved with a subset of the 40, *e.g.*, the housekeepers that rise or fall together by the same amount in the raw data. Algorithms like geNorm[26] and NormFinder[27] can provide a principled means to choose the best housekeepers from among the 40 candidates. In addition to their consistent patterns of expression, these genes were chosen based on their ability to provide coverage of the wide range of expression levels typically observed in experimental datasets.

Using the PanCancer Immune Profiling Panel

Expression profiling is a powerful tool that can be used for many purposes, including identifying relevant immune resistance mechanisms in the tumor microenvironment[28] and developing predictive biomarkers for active immunotherapy[29, 30]. The examples below provide an outline for how the PanCancer Immune Profiling Panel can be used to address these important needs in cancer research and beyond. A study in colorectal cancer demonstrates how the PanCancer Immune Profiling Panel and the PanCancer Pathways Panel may be used together.

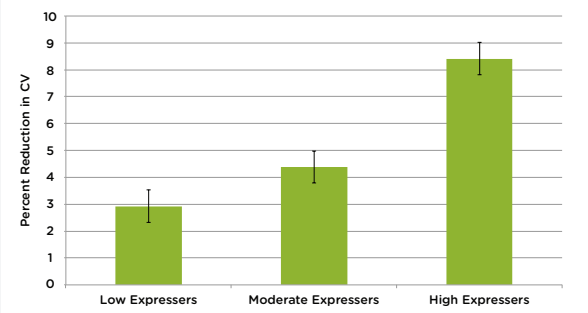

Figure 3: Housekeeping gene normalization results in reduction of expression variance across biological replicates.

Combining Data from the PanCancer Immune Profiling Panel and PanCancer Pathways Panel

Combining data from the nCounter PanCancer Immune Profiling Panel and the nCounter PanCancer Pathways Panel provides an extraordinary window into the biology of cancer. While the Immune Profiling Panel provides a means to assess the response of the host to the presence of a tumor, the Pathways Panel examines the deregulation state of cancer-specific pathways within the tumor. Together, these two panels can provide a holistic survey of a tumor and its microenvironment and serve to enhance insights gathered via classical immunological techniques such as immunohistochemistry and FACS-based approaches as well as mutational status assessed by next generation sequencing. The breadth of genes profiled between these panels (>1300 unique transcripts) enables efficient discovery of high-information-content gene signatures that may ultimately be translated into clinical assays.

In order to illustrate the types of insights capable of being gleaned from combining the PanCancer Immune and Pathways panels, 60 ng of extracted RNA from matched colorectal tumor and normal colon infiltrating immune cells were assayed with both the PanCancer Immune Profiling Panel and PanCancer Pathways Panel. Raw data was imported into nSolver for subsequent normalization and annotation. A subset of the housekeeping genes was used to normalize data for each panel. Upregulation greater than two-fold in tumor samples was observed for 76 genes in the Immune Profiling Panel and 54 genes in the Pathways Panel, respectively; 47 genes in the Immune Profiling Panel and 52 genes in the Pathways Panel were downregulated greater than two-fold.

Expression profiles of tumor samples assayed with the two PanCancer Panels (**Figure 4**) were consistent with previous observations made in colon cancer, where the link between inflammation and cancer is well established[31, 32]. From the PanCancer Immune Profiling Panel, upregulation of innate immune genes—including macrophages, dendritic cells, and antigen processing genes—highlighted the inflammatory response, whereas the increase in checkpoint regulators[19], implied a suppressed adaptive anti-tumor immune response. Common tumor antigens were also detected and suggest that PanCancer Immune Profiling could be used as a method to

identify tumor-specific immune targets. From the PanCancer Pathways Panel, significant upregulation of oncogenes involved with transcriptional regulation, cell cycle and apoptosis, and the mitogen-activated protein kinase (MAPK) cascade were observed. Altogether, these findings demonstrate that combining information about cancer pathways, checkpoints, tumor antigens, and inflammatory cells could be used to establish biomarkers that guide combination cancer therapy.

Comparison of the PanCancer Immune Profiling Panel and Flow Cytometry for Immune Profiling in Chronic Lymphocytic Leukemia

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).[33]

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,

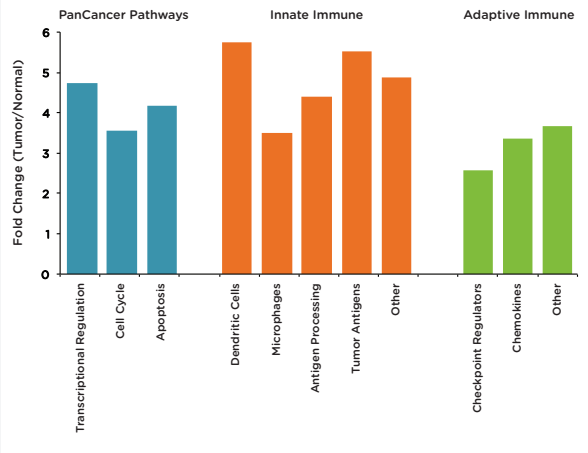

Figure 4 Upregulation of genes associated with indicated activities in tumor samples for both the PanCancer Pathways Panel and the PanCancer Immune Profiling Panel (Innate and Adaptive Immune response).

the two flow cytometry panels identified seven populations: B cells, total T cells, CD8+ T cells, CD4+ T cells, total NK cells, NK CD56dim 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 patients 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 PanCancer Immune Profiling Panel 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

Elucidating the Enhanced effect of MEK Inhibition on Oncolytic Virus Immunotherapy

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.[34] Small-molecule inhibitors of BRAF and MAPK kinase (MEK) in treatment-naive patients with 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 therapeutic 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). Collectively, these data provide preclinical rationale for triple-combination treatment of T-VEC, MEK inhibition, and PD-1 blockade in patients with melanoma.

Using the PanCancer Immune Profiling Panel to Study Autoimmunity

In addition to being used to profile the immune response to cancer, the PanCancer Immune Profiling Panel has been used extensively in cases where a thorough analysis of the innate and adaptive immune response is warranted, as in this next example regarding autoimmunity.

While it is well established that Toll-like receptors TLR7 and TLR9 are both implicated in the activation of autoreactive B cells and other cell types associated with systemic lupus erythematosus (SLE) pathogenesis, paradoxically TIr9 KO mice develop more of a severe disease, indicating a negative feedback mechanism. To investigate this further, the authors developed an inducible rapid-onset murine model of systemic autoimmunity that depends on T cell detection of a membrane-bound OVA fusion protein expressed by MHC class II cells, expression of TLR7, expression of the type I IFN receptor, and loss of expression of TLR9.[35]

100 ng of RNA from mouse skin biopsies was profiled using the mouse and human PanCancer Immune Profiling panels. The group followed up with validation work in human samples by comparing gene expression profiles of lesioned skin obtained from subjects with lupus or psoriasis to that of healthy controls.

Results demonstrated the strength of NanoString as a discovery tool and a lot of overlapping genes were deregulated in both the human and mouse model. Many of the interferon stimulated genes and chemokines highly upregulated in the mouse model were also upregulated in human lupus lesions and, to a lesser extent, in psoriasis lesions, further supporting the relevance of the mouse model to human lupus-like symptoms. Importantly, of all the genes investigated, FasL was found to be the key effector mechanism in the skin in mouse models, as the transfer of FasL-deficient DO11gld T cells completely failed to elicit overt skin lesions. FasL was also upregulated in human cutaneous lupus erythematosus (CLE) biopsies. Overall, the authors' model provides a relevant system for exploring the pathophysiology of lupus as well as the negative regulatory role of TLR9.

Conclusion

The immune system is the body's natural defense against cancer. This system is a complex collection of intricate pathways interwoven with dozens of different cell types that activate or inhibit a response to disease or infection. A previous white paper, Multiplexed Cancer Pathway Analysis on Using the nCounter PanCancer Pathways Panel, described how cancer can be defined using 13 separate canonical pathways influenced by driver genes that regulate cell division and cell fate. It is known that cancer must evade the immune response in order to survive[2,4,6], and the complex interactions between tumors and their microenvironment remain to be elucidated[13]. Researchers studying both cancer and the immune system are challenged with the complexity of understanding their interaction. In the present white paper we have discussed the importance of identifying immune cell types that infiltrate the tumor microenvironment, the role tumor antigens play in initiating the Cancer Immunity Cycle, and outlined the need to assess the entire spectrum of immunological function and responses.

The nCounter PanCancer Immune Profiling Panel is a unique gene expression tool that covers many important features of the immune response in the tumor microenvironment to facilitate rapid development of gene expression profiles across any cancer type. The panel and the nCounter system are ideally suited for use with clinically relevant samples such as FFPE tissue, PBMCs, whole blood, cell lysates, urine, and saliva. Selected genes were specifically chosen to identify and elucidate the complex immunological responses that occur in cancer and in response to external stimuli including immunotherapies. We also showed that when used in conjunction with the PanCancer Pathways Panel, the PanCancer Immune Profiling Panel can deliver insights into the biology of both a tumor and the immune response. Our example highlights the ability of these panels to evaluate the immunosuppressed, highly proliferative, and genomically unstable nature of a colorectal cancer sample.

With contributions from several scientists, we have also touched upon many ways the PanCancer Immune Profiling Panel can be used, including profiling immune cells in chronic lymphocytic leukemia, elucidating the enhanced effect of MEK inhibition on oncolytic virus immunotherapy, and studying the pathophysiology of lupus. The PanCancer Immune Profiling Panel may yet have additional uses to further our understanding of how the immune system responds to cancer and its treatment.

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