

# # 1693 Multi-National, Multi-Center Collaboration to Develop a Novel Gene Expression Tool for Comparative Translational Oncology

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## Abstract

Harnessing the immune system to eliminate cancer and prevent its recurrence is proving to be a powerful therapeutic approach that has achieved unprecedented successes in hematological malignancies and some solid malignancies with a high tumor burden. Next generation immune therapies to improve clinical responses and widen the reach of immunotherapy are being designed at a rapid pace, and informative pre-clinical testing of these approaches can be greatly facilitated using immune competent animals with spontaneous tumors. Pet dogs are immunologically outbred, immune competent and develop spontaneous tumors such as non-Hodgkin's lymphoma, glioblastoma, osteosarcoma, urothelial carcinoma and melanoma that share remarkable clinical, biological and genetic features with their human counterparts. As such, pre-clinical testing of immune therapeutic approaches in dogs with cancer promises to accurately inform human clinical trial design. For this comparative approach to provide maximum information to accelerate human clinical translation of next generation immunotherapies and identify correlative biomarkers of therapeutic response, it is necessary to develop research tools for deep interrogation of the canine immune response. Here we present work conducted through a year-long, multi-center, global collaboration resulting in the creation of a novel gene expression tool for studies of the immune response in dogs treated with immuno-oncology and targeted therapies. This original approach utilizes NanoString's nCounter<sup>®</sup> platform and is termed the Canine IO Panel both described here in this poster.

## Comparative Canine Oncology

The Canine IO Panel has been uniquely designed with 800 canine genes for pan-cancer immune response studies. The panel represents a companion to the widely recognized nCounter Human IO 360<sup>™</sup> and Human PanCancer Immune Profiling panel currently in use with human clinical trials, with significant overlapping content designed for directly comparing human and canine immune response. The customizable panel segments genes into 8 core components including: Cytokine & Chemokine Signaling, Interferon Signaling, Checkpoint Signaling, Complement Cascade, Immune Cell Abundance, Tumor Immunogenicity, Inhibitory Tumor Mechanisms, and Stromal Factors. Genes were selected based on their relevance for the study of oncology, their importance in human clinical studies as well as canine expression profiles from both RNA-Seq and nCounter experiments. Additionally, the canine reference transcriptome, based on CanFam3.1, was utilized for designing the probes; the known genomic variability of dogs in addition to the nCounter hybridization chemistry result in compatibility across a variety of breeds. This new Canine IO panel, used in conjunction with the nCounter platform and correlated with the abundance of data that exist on the platform for similar human IO studies, creates a powerful tool for researchers undertaking comparative human and veterinary studies aiming to develop and improve the understanding and treatment of both canine and human cancers.



Immune					Tumor		Microenvironment
Cytokine & Chemokine Signaling	Interferon Signaling	Checkpoint Signaling	Complement Cascade	Immune Cell Abundance	Tumor Immunogenicity	Inhibitory Tumor Mechanisms	Stromal Factors
305 Genes	47 Genes	67 Genes	33 Genes	52 Genes	74 Genes	94 Genes	101 Genes
Cytokine and Chemokine Signaling	Interferon Signaling	Costimulatory Signaling	Complement System		Antigen Presentation	Epigenetic Regulation	Angiogenesis
					DNA Damage Repair	Hypoxia	Matrix Remodeling and Metastasis
						TGF-beta Signaling	
						Wnt Signaling	

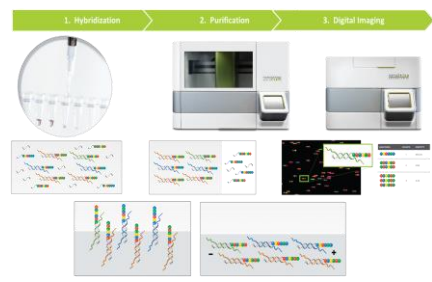
## Selected References Used in Panel Development

1. Dow S. A Role for Dogs in Advancing Cancer Immunotherapy Research. *Front. Immunol.* 2020
2. Dhawan D., et al. Naturally-occurring canine invasive urothelial carcinoma harbors luminal and basal transcriptional subtypes found in human muscle invasive bladder cancer. *PLoS Genet.* 2018
3. Danaher P., et al. Gene expression markers of Tumor Infiltrating Leukocytes. *JITC* 2017
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## Conclusions and Next Steps

The era of effective cancer immunotherapy represents a major change in how cancer is treated, and canine cancer patients undoubtedly have an opportunity to play an important role in advancing this field. The value of using canine cancer patients as a pre-clinical model for immunotherapy has been demonstrated previously, with the best example being the essential role played by dogs with osteosarcoma in the development of the non-specific immunotherapeutic L-MTP (liposomal muramyl tripeptide) as an approved immunotherapy for pediatric osteosarcoma. Immune competent dogs with spontaneous cancers offer an under-utilized opportunity to provide information on safety, efficacy and correlative biomarkers of response to next generation immunotherapies – accelerating their translation into human clinical trials. Development of comparable tools for deep immune profiling of the canine immune response will further enable this valuable model. Procuring adequate drug supplies and reagents for large animal studies is also essential. Finally, broad collaborations will always advance the field more effectively than single institution studies, particularly in situations where essential reagents must be shared or where access to patients with certain tumor types is limited. The best possible outcomes will be studies where the results can be translated promptly to benefit both dogs and humans, with their shared tumor types and strong bonds.

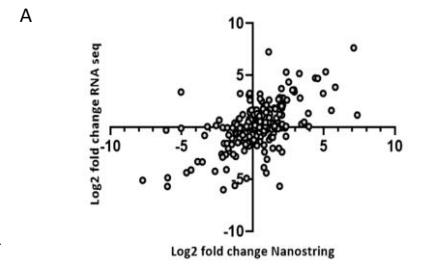
## NanoString Technology & nCounter Workflow



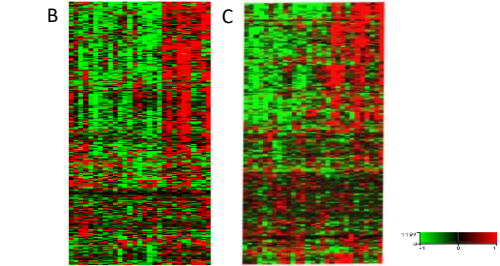
NanoString's nCounter Analysis System performs a highly multiplexed, digital quantification of up to 800 genes in a single reaction. This is achieved via reporter cosedets, which are color-coded "barcodes" specific for each gene. Workflow consists of three major steps: 1) Hybridization, 2) Purification, and 3) Digital Imaging. In the hybridization step, sample material is mixed with the cosedet which hybridizes to the mRNA target in solution. Purification is carried out robotically, which removes excess cosedet and immobilizes the cosedet/RNA complexes in the nCounter cartridge for data collection. CCD capture technology is used for data collection and digital images are processed and reporter probe counts are tabulated for data analysis using NanoString's nSolver<sup>®</sup> software and advanced analysis modules.

Feature	Specification
Number of Targets	Up to 800 targets
Sample Input (Standard (No amplification required))	25-300 ng
Sample Types	PBMC, FFPE-derived RNA, total RNA, fragmented RNA, cell lysate, sorted cells, whole blood/plasma, synovial fluid
Customizable	Add up to 30 unique genes with Panel-Plus and up to 10 custom protein targets
Time to Results	Approximately 24 hours
Data Analysis	nSolver <sup>™</sup> Advanced Analysis Software (RUI)

## Efficient Transition from RNA-Seq to nCounter for Canine Translational Oncology



**Figure A** Comparison of gene expression between T zone lymphoma and normal CD8+ T cells. Two separate experiments using RNA from different cases and controls are shown. In the first, a NanoString panel of 305 genes was applied to 13 cases of CD8+ T zone lymphoma and CD8+ T cells purified from the lymph nodes of 5 healthy dogs. The log2 fold change in gene expression between the average of cases and controls was determined and each gene plotted on the X axis. In a separate RNA-seq experiment using 7 different cases of CD8+ T zone lymphoma and 3 CD8+ T cell controls, the log2 fold change between cases and controls was calculated for the genes used in the first NanoString study y-axis, R<sup>2</sup> between the two methods is .332, p < .001.



**Figure B and C** Interrogation of RNA-seq data from 29 canine invasive urothelial carcinomas (InvUC<sup>2</sup>) for immune cell enrichment. The RNA-seq data were interrogated using an established list of genes that classify human bladder cancer as T cell inflamed (enriched genes indicated in red) or non-T cell inflamed defined by Sweis et al., *Cancer Immunol. Res.* 2016 (B), and using the genes in the canine IO panel (C). Each column represents data from one case. Note that the results with the two gene panels were similar with the same subset of 10 cases (see right side of both heatmaps) being especially T cell inflamed.

## Acknowledgments

The authors would like to thank the National Cancer Institute Cancer Moonshot Program for their support of the Precinct Consortium and Canine Comparative Oncology studies.

