# Interrogation of Spatial Transcriptomics on Canine Tumor and Normal Tissue

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## Abstract

Combination therapy to treat hematological and solid malignancies, including chemotherapy, radiation, targeted and immunotherapy, all hold huge potential for eliciting clinical responses. Informative pre-clinical testing of these approaches can be greatly facilitated using immune competent animals with spontaneous tumors. Most 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 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 novel combination therapies and identify correlative biomarkers of therapeutic response, it is necessary to develop research tools for deep interrogation of the canine tumor microenvironment (TME). Here, we present spatial transcriptomic analysis of multiple canine tumor and tissue types using the GeoMx® digital spatial profiler (DSP) Canine Cancer Atlas panel.

## GeoMx Canine Cancer Atlas

Formalin-fixed paraffin-embedded (FFPE) slides or tissue microarrays were used to profile tumor and normal tissue from canines. Each slide was stained with tissue specific immunofluorescent antibodies, including pan-cytokeratin (PanCK), CD45, Vimentin, IBA1, and CD3.

Regions of interest were selected to assess the TME and normal tissue for a comparison as possible. Tissue slides were then run on the DSP instrument using standard DSP methods. We leveraged the Canine Cancer Atlas panel that contains 1,962-canine-specific genes for transcriptomic profiling.
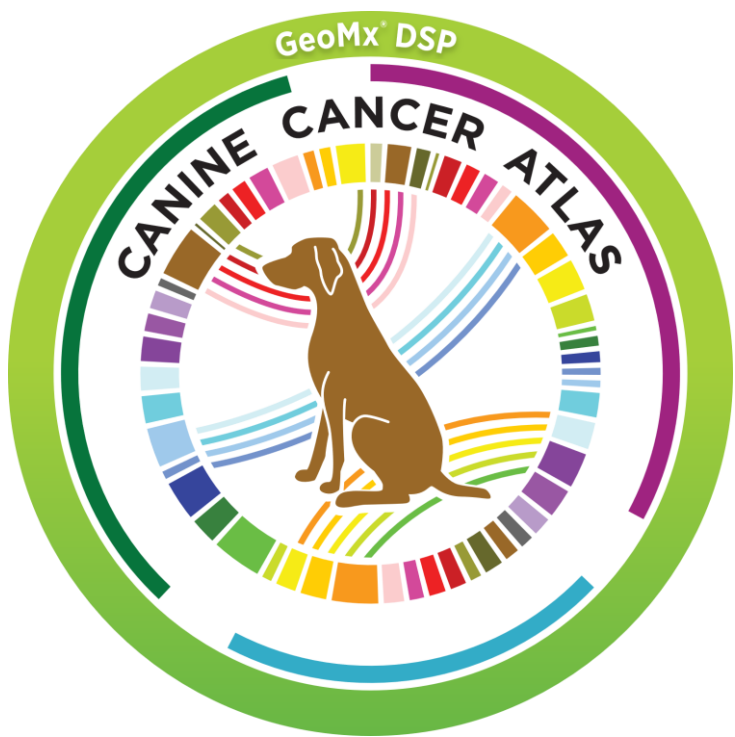


**Figure 1. GeoMx Digital Spatial Profiling with the Canine Cancer Atlas enables quantification of 1,962 protein-coding genes in precisely defined regions of interest. The spike-in up to 400 custom RNA targets is also available to add-in probes for non-coding RNA, synthetic DNA, or exogenous genes.**

## Methods for Transcriptomic Profiling using the GeoMx Canine Cancer Atlas



**Figure 2. GeoMx Workflow with NGS Readout for the Canine Cancer Atlas.**

## Gene Coverage and Pathways in the GeoMx Canine Cancer Atlas

| Adaptive Immunity | | Cell Function | | Immune Response | | Innate Immunity | | Metabolism | | Physiology & Disease | | Signaling Pathways | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| B Cells | 35 | Apoptosis | 121 | Chemokine Signaling | 121 | Complement System | 48 | Amino Acid Synthesis & Transport | 46 | Angiotensin System | 5 | AMPK | 48 |
| BCR Signaling | 78 | Autophagy | 64 | Cytotoxicity | 6 | Dendritic Cells | 22 | Arginine Metabolism | 14 | Cancer Type Relevant | 208 | Androgen | 34 |
| Cancer Antigens | 3 | Cell Adhesion & Motility | 207 | IL-1 Signaling | 63 | DNA Sensing | 46 | Fatty Acid Oxidation | 7 | Circadian Clock | 26 | EGFR | 20 |
| MHC Class I Antigen Presentation | 61 | Cell Cycle | 167 | IL-17 Signaling | 50 | Glycan Sensing | 59 | Fatty Acid Synthesis | 5 | Drug Resistance | 6 | ERBB2 | 24 |
| MHC Class II Antigen Presentation | 18 | Cilium Assembly | 8 | IL-2 Signaling | 39 | Host Defense Peptides | 19 | Glutamine Metabolism | 9 | Matrix Remodeling and Metastasis | 61 | Estrogen | 89 |
| T Cells | 90 | Differentiation | 250 | IL-6 Signaling | 19 | Inflammasomes | 9 | Glycolysis & Glucose Transport | 28 | Neuroendocrine Function | 7 | FGFR | 42 |
| T-cell Checkpoints | 27 | DNA Damage Repair | 92 | Immune Exhaustion | 20 | Myeloid Inflammation | 104 | Glycosylation | 12 | | | FoxO | 84 |
| TCR Signaling | 109 | EMT | 108 | Interferon Response Genes | 29 | Neutrophil degranulation | 120 | IDH1/2 | 10 | | | GPCR | 168 |
| TH1 Differentiation | 23 | Endocytosis | 58 | Lymphocyte Regulation | 89 | NK Activity | 93 | Lipid Metabolism | 95 | | | Hedgehog | 46 |
| TH17 Differentiation | 42 | Epigenetic Modification | 177 | Lymphocyte Trafficking | 47 | NLR Signaling | 82 | Mitochondrial Metabolism / TCA | 55 | | | HIF1 | 79 |
| TH2 Differentiation | 21 | Immortality & Stemness | 33 | NF-κB Signaling | 115 | RAGE Signaling | 8 | Nucleotide Synthesis | 8 | | | Insulin | 84 |
| TH9 Differentiation | 11 | Ion Transport | 42 | Other Interleukin Signaling | 183 | RNA Sensing | 60 | Pentose Phosphate Pathway | 7 | | | JAK-STAT | 123 |
| Treg Differentiation | 15 | Lysosome | 16 | Prostaglandin Inflammation | 4 | TLR Signaling | 136 | Tryptophan & Kynurenine Metabolism | 8 | | | MAPK | 266 |
| | | Oxidative Stress | 164 | TNF Signaling | 94 | | | Vitamin & Cofactor Metabolism | 23 | | | MET | 36 |
| | | Phagocytosis | 100 | Type I Interferon Signaling | 47 | | | | | | | mTOR | 122 |
| | | Proteotoxic Stress | 19 | Type II Interferon Signaling | 42 | | | | | | | Myc | 27 |
| | | RNA Processing | 34 | Type III Interferon Signaling | 8 | | | | | | | | |
| | | Senescence | 131 | | | | | | | | | | |

| Signaling Pathways (cont.) | |
|---|---|
| NO | 9 |
| Notch | 83 |
| p53 | 76 |
| PDGF | 33 |
| PI3K-Akt | 252 |
| PPAR | 20 |
| Purinergic | 5 |
| Retinoic Acid | 5 |
| TGF-beta | 107 |
| VEGF | 71 |
| Wnt | 137 |
| Hippo | 8 |

## GeoMx Profiling of Multiple Normal and Cancer Canine Tissues

**Normal Colon**

**Osteosarcoma**



**Lymph Node**

**Normal Brain**



**Hemangiosarcoma**

**Astrocytoma**



**Figure 3. GeoMx spatial analysis based on tissue morphology, tumor microenvironment, and immune infiltrated hotspots in various tissue types.**

## Integrated Individual Gene and Pathway Built-In Analysis



## Summary and Conclusions

We were able to spatially detect over 1,700 genes across multiple tissue types from canines, including osteosarcoma, glioblastoma, melanoma and normal tissues. Genes were detected in spatial compartments including malignant tumor, tumor stroma and normal tissue.

Together, the GeoMx Canine Cancer Atlas allows for interrogation of the TME of multiple tumor types and has the potential to inform spatial biomarkers for response to therapy, as well as translate the effectiveness of these therapies to humans.

## Acknowledgements

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