

## Of Dogs and Men. Tracing Immune Checkpoint Signatures Across Cancers and Unleashing the Potential of Canine PD-1 Antibodies

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Dogs, sharing genetic similarities and lifestyle factors with us, develop cancers mimicking human ones, yet lack access to the most advanced treatments. Here, I present a canine 'research toolbox,' featuring antibodies, data, and methods to bolster studies into spontaneous canine cancers as a comparative oncology model.

Human immunotherapy employs monoclonal antibodies (mAbs) to block immune checkpoints (ICs) – immuno-modulatory proteins and downstream pathways that are hijacked by cancer cells as a means of immune evasion. Despite the increasing interest in the development of similar drugs for dogs, little was known about the abundance of ICs – the very targets – in canine malignancies. My study bridges that gap by profiling 44 IC genes across 14 canine cancers. Utilizing correlation-driven distance and hierarchical clustering, I identify gliomas and certain sarcomas as prospective research models characterized by strikingly similar IC landscapes across both species. Additionally, I pinpoint IC genes driving inter-species differences.

Human ICs and their impacts are studied deeply - typically in isolation. Here I introduce a broader perspective through characterizing comprehensive IC abundance patterns of particular diagnostic labels and individual human patients alike. In this study I reveal those patterns reflect cancer's histological type, primary site, and inter-patient variation. In certain cancer types the patterns are highly diverse across individuals, pointing to the need for personalized treatment selection and offering clues into the mechanisms underpinning therapy resistance.

To further enable IC-focused studies in dogs, I also characterize two novel mAbs against canine PD-1 receptor – a prominent IC; both exhibit sub-nanomolar affinity, effectively block the key PD-1/PD-L1 interaction and prove useful in a palette of key molecular assays. These antibodies represent valuable research and diagnostic tools as much as potential veterinary therapeutics.

To address the risk of severe immune reactions to murine-derived antibodies if used in dog patients, I develop a specialized set of 'caninization' methods and canine mAb sequence libraries, resulting in de-immunized, dog-like PD-1 antibody constructs. These advancements can pave the way for canine clinical trials and streamline veterinary antibody development.