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The Italian-Canine Cancer (ICC) Biobank: our 10-year challenge

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The role of pet dogs in comparative oncology and the advantages of this model over rodent and in vitro models for studying cancer biology and accelerating the development of novel anti-cancer therapies are now evident and supported by many research papers. Aside the opportunity to test new compounds in dogs to assess toxicity and safety, drug companies are now considering the veterinary medicine market for drugs that do not reach clinical trials in human medicine. The increasing knowledge related to the pathogenesis and biology of the most frequent cancers in dogs will also permit to identify with more accuracy the ideal canine model candidate for testing new treatments.

In the past five years, next generations sequencing techniques and the complete annotation of the canine genome have expedited this process. Within this context, canine lymphoma, melanoma, hemangiosarcoma, osteosarcoma and head and neck squamous cell carcinoma are the most relevant tumors both in Europe and USA. The transcriptomes, mutation profiles and methylomes of these cancers have been investigated revealing important data that are now used for drug testing, genetic selection and prognostic considerations.

Given the increasing importance of collecting tumor tissues with high quality preservation to apply different “omics” platforms in veterinary medicine, few canine cancer tissue banks have been settled down in USA, but none in Europe. By definition, cancer tissue banks represent facilities organized to collect, store and distribute samples of tumors and normal tissues to be used in basic and translational cancer research. The process beyond the establishment of cancer tissue banks necessitates a tight integration between medical oncologists, surgeons and pathologists. Even if standard operation procedures are not described in veterinary medicine, the local Ethical Committee considers mandatory the informed consent obtained by owners to use tissues and liquids for research purposes.

The general workflow starts with the collection of cancer tissue during or after surgery. This is done by a surgeon, who supervises the cutting procedure and stores, as quickly as possible, the sampled tissue at low temperature in RNA-later (-20 C) or frozen without solutions (-80 C). Both methods guarantee preservation of proteins and nucleic acids integrity, storage for long periods, and shipment worldwide to research labs by maintaining the cold chain.

A general issue to consider during this phase is to preserve the integrity of the tumor specimen, always giving priority to establish the histopathological diagnosis and evaluate surgical margins. This material should be fixed in formalin for hematoxylin and eosin staining. The diversity of sample types to be collected depends on the specific research needs and the cancer histotype. For instance, in the case of canine lymphoma, it is fundamental to obtain tumor tissue from nodes or other infiltrated organs; notably, the storage of bone marrow and peripheral blood is also highly appealing to conduct investigations on lymphoma cells migration. Conversely, for other tumors that are known to use different mechanisms of spread, samples obtained from metastatic organs would be more important to elucidate the progression pathways. Given the increasing importance of serial liquid biopsies in many cancers both for the diagnostic and follow-up purposes, urine and body fluids other than peripheral blood are highly demanded.

Somatic mutation profiling using massively parallel next-generation sequencing is one of the most prevalent application of precision medicine, and one of the major issues when applying this technology in veterinary medicine to identify canine cancer mutations is related to the extensive DNA diversity among pure breeds or the canine population in general. Indeed, the presence of germ-line variants complicates the effort to identify targetable “driver” mutations. For this reason, when conducting somatic mutation profiling, it becomes necessary to sequence both tumor and normal samples in parallel to identify those variants that are specific to the cancer and hence, more

likely to be related to the cancer phenotype. Especially for pure canine breeds that are predisposed to cancer or other diseases, a predisposing variant is present in the “normal” DNA, either inherited or as an early postzygotic event. Many human sequencing centers use matched tumor-normal tissues to facilitate more accurate calling of somatic mutations by using the normal DNA to exclude germline variants from the tumor calls and this should be highly recommended in future veterinary medicine researches.

The Italian-Canine Cancer (ICC) Biobank was started in 2009, aimed at collecting samples of canine lymphoma and normal tissue (skin), both frozen and FFPE, as well as whole blood and bone marrow and with a owners’ full consent. The ICC Biobank was initially developed by one oncologist (LM) and one pathologist (LA) for the research needs of specific projects, thereby opening a collaboration between private and public institutions. Over the years, the data associated with stored anonymized biospecimens have increased in complexity from basics, such as date of collection signalment, histological diagnosis, clinical stage, treatment, treatment response and outcome, to extensive information sets encompassing many aspects of genetic, proteomic, and other “omics” information. Currently, the ICC Biobank comprises more than 350 canine lymphoma samples including peripheral blood (serum and plasma), bone marrow, paraffin and frozen tumors, and DNA germline from skin punch biopsies, to which researchers worldwide can apply for samples and/or data to conduct cancer-related research.
Starting from 2017, we have expanded our interest by collecting samples from dogs with melanoma, osteosarcoma, leukemia, hemangiosarcoma and from cats with squamous cell carcinoma or sarcoma, considering the high relevance of these cancers both in veterinary oncology and translational medicine. The ICC Biobank represents now the largest Italian multi-centre veterinary collection facility, collecting high-quality tissues from canine donors coming from public and private veterinary clinics. The mission of ICC Biobank is to make tumor specimens available not just to academic researchers but to all who are actively engaged in veterinary cancer research. The major aim is to speed up the understanding of the biology of canine cancer, possibly leading to the discovery of new therapies, thereby improving veterinary care and patients’ outcome and starting comparative oncology programs.