Clinical, Pathological, and Ethical Considerations for the Conduct of Clinical Trials in Dogs with Naturally Occurring Cancer: A Comparative Approach to Accelerate Translational Drug Development

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Abstract

The role of comparative oncology in translational research is receiving increasing attention from drug developers and the greater biomedical research community. Pet dogs with spontaneous cancer are important and underutilized translational models, owing to dogs' large size and relative outbreeding, combined with their high incidence of certain tumor histotypes with significant biological, genetic, and histological similarities to their human tumor counterparts. Dogs with spontaneous tumors naturally develop therapy resistance and spontaneous metastasis, all in the context of an intact immune system. These fundamental features of cancer biology are often lacking in induced or genetically engineered preclinical tumor models and likely contribute to their poor predictive value and the associated overall high failure rate in oncology drug development. Thus, the conduct of clinical trials in pet dogs with naturally occurring cancer represents a viable surrogate and valuable intermediary step that should be increasingly incorporated into the cancer drug discovery and development pipeline. The development of molecular-targeted therapies has resulted in an expanded role of the pathologist in human oncology trials, and similarly the expertise of veterinary pathologists will be increasingly valuable to all phases of comparative oncology trial design and conduct. In this review, we provide a framework of clinical, ethical, and pathology-focused considerations for the increasing integration of translational research investigations in dogs with spontaneous cancer as a means to accelerate clinical cancer discovery and drug development.

Key words: cancer; canine; clinical trials; comparative oncology; drug development; pathology

Introduction

Over the last 2 decades, the field of comparative medicine, as it pertains to the study of animals with naturally occurring disease, has witnessed significant gains in momentum and visibility in the biomedical research community. However, the concept of using animals with spontaneous diseases as surrogates for

understanding mechanisms of disease pathogenesis in humans is almost a century old. It was Nobel laureate August Krogh who, in 1929, first proposed the idea of naturally occurring animal diseases as models for human diseases, advocating for “laboratories of comparative physiology” and stating that “for a large number of problems there will be some animal of choice on

which it can be most conveniently studied.¹¹ These principles initially set forth by Krogh now embody the sentiment of an expanding network of scientists, physicians, veterinarians, and drug developers who realize the translational value of spontaneous, large (companion) animal models of disease.

Undoubtedly, the reagent-rich, highly reproducible, and genetically manipulable aspects of traditional laboratory animal species have allowed for significant biomedical research discoveries to be made in disease pathogenesis and associated therapeutic interventions.²⁻⁴ Nonetheless, the limits of these inducible or genetically engineered disease models are increasingly becoming apparent. For example, the proportion of anticancer therapies that fail during expensive phase III trials is substantial, with recent estimations suggesting that only 10% of agents entering clinical cancer trials make it to US Food and Drug Administration (FDA) approval.⁵⁻⁷ This high failure rate is primarily attributed less frequently to issues of drug safety but more so to a lack of effectiveness in the clinical setting, calling into question the predictive value of current preclinical *in vivo* efficacy studies on patient response and outcome.^{6,8,9} Thus, it is becoming more apparent that this disconnect between preclinical and clinical efficacy is at least in part due to an inability of laboratory animal models to fully recapitulate the complex genetic, biological, and environmental factors driving disease phenotype.

Insightful and foundational work on the comparative biology, pathology, and molecular aspects of naturally occurring diseases in companion animals has identified a considerable number of conditions that parallel a human disease equivalent and have strong potential to serve as informative translational research models.¹⁰⁻²⁰ Although not comprehensive, this list (summarized in Table 1) encompasses a diverse range of companion animal diseases spanning from cancer to cardiomyopathy, diabetes and osteoarthritis, to ocular autoimmunity, muscular dystrophy, and acute spinal cord injury.^{10-13,21-24} While still an underutilized research resource, it is quickly being recognized that these natural disease models likely exhibit more significant overlap, in terms of their shared environmental exposures, underlying pathophysiology, and response to therapeutic interventions, with their human disease counterparts.^{3,25} Additionally, the rapid growth and specialization of clinical veterinary medicine has shifted paradigms such that similar to human medicine, utilization of state-of-the-art diagnostics and administration of standard of care therapeutic regimens is status quo for many companion animal diseases treated at academic veterinary medical centers. Thus, physicians, veterinarians, and scientists, leveraging a complementary in-depth knowledge of the comparative pathophysiological aspects of disease states in both humans and animals, are increasingly conducting collaborative and informative translational research in companion animals with certain naturally occurring diseases.

For example, the American Veterinary Medical Association (AVMA) maintains an Animal Health Studies Database website (Table 1; https://ebusiness.avma.org/aahsd/study_search.aspx), which, at the time of drafting this manuscript, listed approximately 340 clinical trials in companion animals, ranging from evaluation of mesenchymal stem cell therapy for cats with chronic kidney disease and dogs with osteoarthritis, to immunotherapy for equine skin tumors and gene therapy for canine degenerative myelopathy, a disease that shares biological, histological, and genetic similarities with amyotrophic lateral sclerosis in humans.¹⁴ In many cases, these studies encompass both investigator-initiated and industry-sponsored trials and represent a cross-disciplinary collaboration between academic

veterinary and human clinicians and scientists, providing a prime example of the value in the One Health Initiative.²⁶

Comparative Oncology

An estimated greater than 1 million new cases of canine cancer are diagnosed each year in the United States; in a recent retrospective study indexing causes of canine mortality, cancer is reported to be the leading cause of death, with estimated canine cancer mortality rates of around 30%.^{10,27,28} This strikingly large cancer burden in dogs highlights a population of spontaneously arising tumors, many which are of comparatively relevant histologies to human tumors including non-Hodgkin lymphoma, melanoma, osteosarcoma, and bladder carcinoma, and whose treatment may be incorporated into cancer drug development strategies as an intermediary to human clinical trials.^{10,29}

While receiving recently renewed attention from the larger biomedical research community over the last 1 to 2 decades, the field of comparative oncology has long been at the forefront of utilizing naturally occurring diseases in companion animals to conduct informative translational research. Clinical trials in dogs with spontaneous tumors were being conducted as early as the mid 1970s.^{30,31} The natural occurrence of cancer in pet dogs affords many disease attributes that are advantageous over inducible and genetically engineered animal models of cancer. Dogs share common environmental exposures and similar genetic, anatomical, and physiological make-up to humans.^{10,27,29,32} They develop tumors on a more chronologically relevant scale and under the selective pressure of an intact immune system, allowing for the development of intra-tumoral heterogeneity and thus a natural evolution of the fundamental processes of immune evasion, therapy resistance, and metastasis. Yet compared with human clinical studies, the comparatively shorter life span of dogs allows for more rapid attainment of study endpoints and efficacy data.²⁹

These attributes uniquely position the dog as a translational animal model with significant potential to complement our understanding of human cancer biology and inform oncology drug development.^{10,27,32} Notable prior work by Withrow and colleagues, who pioneered techniques for human limb-sparing surgery in dogs with osteosarcoma,³³ and other groups such as Kurzman, MacEwen et al, and London et al, with seminal work in development and clinical evaluation of immunotherapy for osteosarcoma³⁴ and molecular-targeted therapy for oncogene (c-kit) driven canine mast cell tumor (MCT),³⁵ respectively, have exemplified this potential. A more complete list summarizing the contributions of comparative oncology trials to translational research and the development and approval of anticancer therapeutics is listed in Table 2. Here, we provide further insights, in terms of clinical, pathological, and ethical considerations, for the increasing utilization of translational research investigations in dogs with spontaneous cancer.

Role of the Pathologist in Supporting Comparative Oncology Research and Clinical trials

The discovery of cancer driver mutations and the associated rise in the development of molecularly targeted agents over the last decade has resulted in a significant increase in the role of the pathologist in human oncology.³⁶ As an example, the diagnosis of human breast cancer has evolved from a system primarily based upon tumor histological appearance to one that depends on advanced molecular diagnostics for proper

Table 1 List of Translationally Relevant, Spontaneous Companion Animal Diseases and Associated Active Clinical Trials

Disease Entity	Affected Species	No. Active Trials (AAHSD)	Example of Translationally Relevant Trial (AAHSD)
Cardiovascular			
Hypertrophic cardiomyopathy	Cats	3	Identification of associated genetic mutation (AAHSD004249)
Dilated cardiomyopathy	Dogs	3	Cardiac energy consumption for early screening and diagnosis (AAHSD000164)
Arrhythmogenic right ventricular cardiomyopathy	Dogs	1	Mesenchymal stem cell therapy (AAHSD000193)
Immunologic disorders			
Feline immunodeficiency virus	Cats	0	—
Inflammatory bowel disease	Cats, dogs	4	Effect of a probiotic bacterium on intestinal health of dogs with inflammatory bowel disease (AAHSD000156)
Keratoconjunctivitis sicca	Dogs	5	Evaluation of the safety and efficacy of a novel therapeutic D929 (AAHSD004444)
Recurrent uveitis	Horses	0	—
Metabolic/endocrine			
Type II diabetes	Cats	2	Incretin-based tests for early diagnosis (AAHSD000146)
CKD	Cats	4	Renal intra-arterial allogeneic mesenchymal stem cells for feline CKD (AAHSD000210)
Glomerulonephritis	Dogs	0	—
Aging	Dogs	0	—
Genetic			
Degenerative myelopathy (SOD1-associated ALS)	Dogs	3	Adeno-associated virus mediated SOD1 gene silencing therapy in naturally occurring canine degenerative myelopathy (AAHSD004063)
Duchenne muscular dystrophy	Dogs	0	—
Lysosomal storage disease	Cats, dogs	0	—
Hemophilia	Dogs	0	—
Musculoskeletal/neurologic			
Acute spinal cord injury	Dogs	3	Mesenchymal stem cell therapy in dogs with acute paraplegia due to thoracolumbar disk extrusion (AAHSD000277)
Epilepsy	Dogs	6	The effect of cannabidiol on seizure activity in epileptic dogs (AAHSD004689)
Chronic osteoarthritis	Dogs	15	CryoShot Canine (R1) cell therapy in dogs with osteoarthritis (AAHSD000315)
Cancer			
Osteosarcoma	Dogs	19	Evaluation of a recombinant, attenuated <i>Listeria monocytogenes</i> expressing a chimeric human HER2/neu protein in dogs in the adjuvant setting with osteosarcoma (AAHSD004477)
Soft tissue sarcoma	Dogs, cats	12	Defining PK and biological activity of systemic oncolytic Vesicular Stomatitis Virus (VSV) (AAHSD000101)
Melanoma	Dogs	14	OMX-4.80 (Zox) a novel protein oxygen transporter and radiation sensitizer for dogs with oral melanoma (AAHSD004090)
Non-Hodgkin's lymphoma	Dogs, cats	23	A pilot study of VerdineXor (XP-01 inhibitor) plus RV1001 (PI3K delta inhibitor) for the treatment of canine lymphoma (AAHSD000077)
Urothelial carcinoma	Dogs	4	B-RAF V600E targeted vaccine for female dogs with bladder cancer (AAHSD004644)
Brain tumors	Dogs	11	Molecular Combinatorial Therapy for Canine Malignant Gliomas (AAHSD000005)

AAHSD, AVMA Animal Health Studies Database; CKD, chronic kidney disease; HER2, human epidermal growth factor receptor 2; PK, pharmacokinetics; PI3K, phosphoinositide 3-kinase. SOD1, superoxide dismutase 1; ALS, Amyotrophic Lateral Sclerosis.

subtyping of patients' tumors based on hormone receptor and growth factor receptor (human epidermal growth factor receptor 2) status.^{37,38} The importance of these advances in molecular pathology has been realized through the significant clinical benefit observed as a result of the personalized therapy of individual's tumors via therapeutic targeting of these molecules.³⁹ Moreover, the advent of high-throughput and cost-effective genomic sequencing has fueled an ever-increasing number of investigations into the intrinsic genetic vulnerabilities and gene expression profiles of tumor cells, which continue to uncover

novel molecular targets and pathways across many human cancers, which often already have an associated therapeutic agent in early-phase clinical development.⁴⁰ Thus, the role of the human pathologist in oncology clinical trials has and will continue to become increasingly important for all phases of the design and implementation of clinical investigations evaluating these novel therapeutic strategies.

For example, the potential success of many of these investigative therapies will depend on the proper identification of the tumor type and patient subset most likely to respond. In this

Table 2 Completed Comparative Oncology Trials That Have Informed Anticancer Drug Development

Therapeutic Agent	Tumor Type	Pathologist's Role	Canine Clinical Trial Findings	Current Stage of Drug Development	Reference
AAV-phage delivery of endothelial-targeted TNF- α (RGD-A-TNF)	Bone and soft-tissue sarcomas	Evaluation of pre- and posttreatment serial tumor biopsies Immunofluorescent localization of RGD-A-TNF Full necropsy of trial patients	Determination of an optimal/safe dose for RGD-A-TNF Demonstrated selective targeting of tumor vasculature	Phase III trial of a similar tumor-vasculature targeted TNF- α drug ongoing (ClinicalTrials.gov Identifier: NCT01098266)	84
Rapamycin (mTOR inhibitor)	OSA	Histological review of pre- and posttreatment tumor biopsies for tumor necrosis and sample selection guidance for PD endpoints	Pharmacokinetic demonstration of dose-dependent drug exposure Demonstration of PD target modulation	Phase III trials ongoing (ClinicalTrials.gov Identifiers: NCT00879333, NCT00876395) Temsirolimus (rapamycin pro-drug) FDA approved for renal cell carcinoma	85
NHS-IL-12 Immunocytokine	Melanoma	Histological review of pre- and posttreatment biopsies, with semiquantitative scoring of necrosis and inflammation Immunohistochemical analysis and scoring of pre- and posttreatment tumor-infiltrating immune cells	Successfully defined the therapeutic window and determined a safely administered dose of NHS-IL-12 for first in human trials	Phase I trials ongoing (ClinicalTrials.gov Identifiers: NCT01417546, NCT01417546)	58
Iniparib (PARP1 inhibitor)	MelanomaSCC, STS	Histological review of pre- and posttreatment tumor biopsies for quality control and sample selection for tumor PK	Determined safety/tolerability and PK of iniparib as single agent or in combination with other chemotherapies Demonstrated drug does not accumulate in tumor tissues	Failed phase III trials in metastatic triple-negative breast cancer and NSCLC	57,86
Ibrutinib (Bryton tyrosine kinase inhibitor)	B cell lymphoma	Histological & immunohistochemical review of tumor biopsies for confirmation of diagnosis and study enrollment	Proof-of-target occupancy; Demonstration of in vivo anti-tumor activity	FDA-approved for the treatment of mantle cell lymphoma and CLL	87
PAC-1 (procaspase-3 activator)	Lymphomamalignant glioma, metastatic OSA	Assessment of hematologic and biochemical toxicities; Cytologic review of LN aspirates Proof-of-target via retrospective immunohistochemical evaluation of tumor samples	Determined the safety/tolerability and PK of PAC-1 alone and with chemo-radiation; Validation of tumor PD endpoints Demonstration of in vivo antitumor activity	Phase I trials in brain cancer ongoing	88–91
Ganetespib (HSP-90 inhibitor)	Multiple	Histological confirmation of diagnosis prior to trial enrollment Assessment of hematologic and biochemical toxicities	Determined the safety/tolerability, PK, and potential dosing schedules of a novel HSP-90 inhibitor Validation of PD/biomarker endpoints of target modulation	Failed phase III trial in advanced NSCLC (all patients) Recently completed phase II trial in molecularly selected NSCLC patients	92,93
GS-9219/VDC-1101 (nucleotide analog pro-drug)	B cell lymphoma	Histological diagnosis & immunophenotyping prior to trial enrollment Cytologic confirmation of cases of complete response Gross and histological toxicity assessment in normal beagle dogs; evaluation of LN PD endpoints	Determined safety/tolerability, and PK/PD Demonstrated proof-of-concept of the antitumor activity	Phase I/II human trial completed	94,95
Toceranib/sunitinib (multi-kinase inhibitor)	Multiple	Assessment of hematologic and biochemical toxicities Histological confirmation of diagnosis prior to study enrollment	Determined safety/tolerability and PK Proof of target modulation and demonstration of antitumor activity	FDA approved for veterinary and human use	96,97

FDA, US Food and Drug Administration; LN, lymph node; NSCLC, non-small cell lung cancer; OSA, osteosarcoma; PK, pharmacokinetics; PD, pharmacodynamics; SCC, squamous cell carcinoma; STS, soft tissue sarcoma; TNF- α , tumor necrosis factor alpha. AAV, Adeno-associated virus; RGD, arginine, glycine, aspartic acid tri-peptide; mTOR, mammalian target of rapamycin; NHS-IL12, fusion protein comprised of interleukin 12 fused to the heavy chain of the NHS76 monoclonal antibody which binds DNA/histone complexes; PARP1, Poly ADP-ribose polymerase 1; PAC-1, procaspase activating compound 1; HSP-90, heat shock protein 90.

regard, pathologists are critical to the accurate tumor molecular subtyping and patient stratification required for trial enrollment. In addition, the on- and posttreatment evaluation of biomarkers and pharmacodynamic (PD) endpoints of target modulation provides critical insights towards understanding and uncovering predictors of both treatment response and failure. Pathologists provide valuable expertise and insight to all aspects of this process, ranging from evaluation of surgically procured tumor tissues for quality control prior to molecular analysis to guiding the development and interpretation of tissue-based PD assays.

As compared with their human counterparts, there is a significant lack of knowledge regarding the overall genomic landscape and molecular pathogenesis underlying the etiology of common and translationally relevant canine cancers. As a result, the role of the veterinary pathologist in comparative oncology trials remains relatively reduced as compared with that required in human oncology drug trials. In veterinary oncology, the most important and commonly utilized role of the pathologist is to ensure accuracy and quality control in the diagnosis of companion animal tumors required for trial eligibility/enrollment. In addition, it is imperative that the veterinary pathologist ensures that these pretreatment biopsies are accurately graded for known prognostic factors associated with the tumor type being investigated, which typically involve histological grading and subtyping, measures of proliferative indices (mitotic index, Ki67), and subjective evaluation of tumor differentiation. Additionally, at this stage of the trial, veterinary pathologists can also provide important guidance regarding aspects of tissue handling and fixation methods as well as measures of tissue quality (in terms of percentage of necrosis, or normal versus tumor tissue) for tissue banking purposes or prior to use of these samples in genomic assays intended to stratify trial patients.

In contrast to human oncology, at present, there is only one canine tumor type (canine cutaneous MCT) that requires the pathologist to interpret advanced diagnostic assays for purposes of prognostication based on molecular status.^{41–43} These evaluations are based on prior work identifying activating mutations in the *c-kit* oncogene in a subset of dogs with MCT⁴⁴ and include immunohistochemistry for KIT protein localization and PCR for determination of *c-kit* mutation status.⁴⁵ However, even for the case of *c-kit* mutations in canine MCT, the value of *c-kit* mutation status and KIT protein localization as independent prognostic factors remains unclear and may only be useful in differentiating the biologic behavior of cases of histologically low-grade canine MCT.^{46,47} A growing number of retrospective pathological investigations into the expression of actionable molecular targets in other canine cancers, such as osteosarcoma, soft tissue sarcoma, and hemangiosarcoma, are being performed.^{48–52} However, in comparison with human oncology, these studies are few and currently lack the statistical power necessary to inform the clinical management of canine cancer patients and thus are not yet incorporated into the pathological characterization and clinical diagnosis of these tumors.

Nonetheless, the further advancement of veterinary and comparative oncology requires continued evaluation and incorporation of these data obtained from studies on the molecular pathogenesis of canine tumors. In a joint effort, the Veterinary Cancer Society and American College of Veterinary Pathologists (ACVP) have established the Oncology-Pathology Working Group (OPWG), an organization dedicated to promoting the interaction of veterinary oncologists and pathologists to ensure the highest standard of pathology reporting in veterinary

oncology (<http://vetcancersociety.org/vcs-members/vcs-groups/oncology-pathology-working-group/>). Currently, the primary function and objective of the OPWG is to retrospectively collate peer-reviewed, published scientific literature of oncologic pathology investigations in companion animals. This information is used to generate species-specific consensus documents containing standardized recommendations for the use and reporting of tumor grading systems and prognostic and predictive parameters, respectively. However, with this current literature review-based model, the OPWG remains subject to the same inherent issues associated with trying to histologically and molecularly characterize and prognosticate companion animal cancers based on the equivocal results of multiple, small, frequently underpowered studies in veterinary oncology.

Thus, a potential opportunity for improving this model and ultimately the (histological and molecular) subclassification of companion animal neoplasms could arise from the establishment and incorporation into the OPWG of a larger consortium of oncology-focused veterinary pathologists. Ideally, this network of veterinary pathologists would span multiple disciplines and sectors of the profession. To conceptualize how this consortium may function, an initial step may be the continuation of the OPWG literature-based model wherein they provide a consensus document of tumor histotype-specific schemes of prognostic grading and histological and molecular subclassification, which warrant more rigorous validation. Subsequently, this assembled consortium of veterinary pathologists could then be employed to prospectively evaluate this list of literature-based scientific evidence in a single, adequately powered, multi-institutional study utilizing already present and accessible large populations of companion animals receiving standard of care cancer treatment at academic veterinary hospitals.

As is currently the case for the OPWG, to establish a more formalized, expanded, collaborative research group structured around specific tumor histotypes, this endeavor would require joint leadership and participation of oncologists and pathologists of the American College of Veterinary Internal Medicine/Veterinary Cancer Society and the ACVP, respectively. Perhaps this consortium could work through the framework of a larger, already-established governing body such as the ACVP, which could function as a virtual coordinating center for these studies, facilitating input from both veterinary oncologists and pathologists to ensure that these prospective clinical studies are appropriately designed and will indeed validate or disprove current literature-based consensus recommendations. Currently, a paradigm similar to this has been launched for the comparative investigation of canine brain tumors and could serve as a valuable framework for the establishment of similar consortiums focused around other translationally relevant canine tumor types.⁵³ Moving forward, it is imperative that the veterinary medical community continue to work together and strive to set forth guidelines for the implementation of scientifically rigorous studies designed to standardize the molecular and histopathological evaluation of canine tumors to further advance veterinary clinical oncology and comparative oncology research.

So while at present the role of the veterinary pathologist in comparative oncology research and clinical studies remains relatively minimal and unformalized, it should be emphasized that this is not the result of a lack of breadth and depth of research and advanced diagnostic pathology expertise in the veterinary academic community. Translational research is an area of increasing scientific priority that can greatly benefit from the incorporation of the specialized expertise of

veterinary pathologists in both preclinical and comparative investigations. In a national effort to stimulate and advance comparative approaches for translational oncology drug development, the Comparative Oncology Program and associated Comparative Oncology Trials Consortium (COTC) (<http://ccr.cancer.gov/resources/cop/COTC.asp>) was launched in 2004 by the US National Institutes of Health (NIH)-National Cancer Institute (NCI).⁵⁴ Comprised of an institutional network of 20 academic veterinary teaching hospitals across the United States and Canada, the program thus far has completed 12 multicenter clinical trials in pet dogs with spontaneous cancers.⁵⁵ Currently ongoing clinical trials in veterinary oncology listed either in the AVMA online database or through the COTC, and which have the potential to inform human oncology drug development and practice, include trials ranging from oncolytic virus therapy, molecular-targeted agents for metastasis prevention, and immunotherapy for brain tumors to investigations into medical devices for malignant urethral obstruction and cancer-associated pain management (<https://ccr.cancer.gov/Comparative-Oncology-Program/pet-owners/trials>; https://ebusiness.avma.org/aahsd/study_search.aspx).

Many of these trials are supported by the COTC PD core, a virtual laboratory composed of various investigators from COTC member institutions with expertise in areas such as pathology, immunohistochemistry, flow cytometry, and pharmacokinetics.⁵⁶ These laboratories have been selected through an application and ranking process based primarily on expertise and available capacity to provide intellectual study design and PD assay support to COTC trials. As such, veterinary pathologists represent a key team member for the successful administration of the COTC PD core and serve to provide industry sponsors with an important intellectual resource for all stages of a COTC trial. This ranges from early interactions regarding design of appropriate preclinical studies required for initiation of a COTC trial, to input and review on proposed biological endpoints for the trial, all the way through to direct on-trial and posttrial support through providing expertise in aspects such as histopathology, and immunohistochemistry for evaluation of target modulation. A review of previously published COTC comparative oncology trials highlights the value of veterinary pathologists in trial conduct, with their contributions ranging from histopathology review for guiding sample selection for measurement of intratumoral drug levels and target PDs, to scoring of necrosis and T cell, B cell, and myeloid cell tumor infiltrates in pre- and posttreatment biopsies from dogs receiving an IL-12 immunocytokine.^{57,58}

Although these studies clearly demonstrate a working product for pathology support of COTC studies, the incorporation of veterinary pathologists into COTC trial design typically occurs through individual investigator (pathologist) engagement and often is influenced by factors of time availability or particular assay capabilities. As such, the isolated and individualized nature of this virtual laboratory support system opens the door for significant variability, and potential missed opportunity, in the design and conduct of pathology-based study endpoints across both COTC-coordinated and individual institutional trials. While the centralization and establishment of a single, physical COTC pathology core facility specifically designated for comparative oncology studies may improve some of these issues, this solution is financially impractical and still likely leaves intellectual pathology input for COTC trial design to the indiscretion of one, maybe two, veterinary pathologists.

Alternatively, input from a larger team of multi-disciplinary pathologists will allow for committee-based standardization and quality control of pathology-based assays and endpoints to

maximize histological, immunological, and molecular pathology information gained from comparative oncology trials. Establishment of this infrastructure would also be invaluable as clinical trial enrollment and conduct begins to expand outside of academic veterinary medical centers, such as a pilot study that has already occurred through the Colorado Front Range Oncology Group (Regan and Thamm, personal communication). Furthermore, this type of input from the greater veterinary pathology community may perhaps help to identify and address knowledge gaps or secondary hypotheses peripheral to the primary study design, thus also helping to minimize any missed scientific opportunities with these trials. To leverage this up-front, committee-based approach for pathology support of comparative oncology trials, veterinary pathologists could also work towards compiling this data into a centralized, publicly available research resource. For example, tumor histology and immunohistochemistry slides could be digitally scanned and linked with other molecular data and patient clinical information from these trials to begin to create a resource similar to The Cancer Genome Atlas (<https://cancergenome.nih.gov/>) and the associated Cancer Digital Slide Archive (<http://cancer.digitalslidearchive.net>).

Lastly, while immunotherapy is quickly becoming a mainstay of human cancer treatment, the field of veterinary immunoncology remains at a distinct disadvantage in terms of even our basic understanding of the immunological landscape of common canine tumors.⁵⁹ Studies in human oncology demonstrate genomic and immunohistochemical features of the tumor and tumor microenvironment that predict and/or correlate with response to immunotherapy.^{60,61} On the other hand, inducible and genetically engineered mouse tumor models have deficiencies in predicting immunological and molecular determinants of response to immunotherapy, and thus there is increasing interest by NIH-NCI to determine the suitability of the canine model for evaluation of novel immunotherapies (<https://grants.nih.gov/grants/guide/rfa-files/RFA-CA-17-001.html>). Spontaneously occurring tumors in companion animals likely harbor significant potential to inform human immunotherapy trials. However, what is desperately needed are large-scale histological, immunohistochemical, and genomics-based studies characterizing the tumor microenvironment of translationally relevant canine tumors, including measures of canine tumor mutational burden and neo-antigen density, as well as the phenotypic and functional characterization of specific tumor-infiltrating immune cell subsets. If properly conducted, these large-scale descriptive molecular and immuno-pathological studies can provide the veterinary oncology community with the information required for the informed design of canine immunotherapy trials. Thus, the utilization of veterinary pathologists to determine tissue-based predictors of response to immunotherapy in dogs will be critical to determining whether these correlates are similar or different from their human counterparts, and in the end will greatly help to validate (or not) the dog model for evaluation of novel immunotherapeutic combinations.

Clinical Trial Design and Implementation in Veterinary Oncology

There has been a recent increase in interest in the biomedical community in the evaluation of novel cancer therapies in pet animals with spontaneous cancer. This includes treatments designed to be veterinary therapeutics as well as agents for which data generated in tumor-bearing animals may inform human clinical development. Several recent reviews have discussed

veterinary oncology clinical trial design and implementation in detail.^{62,63} The traditional “phases” of clinical trial design and primary goals are summarized in Table 3.

Phase I Trials (Dose-Finding)

The primary goal of phase I trials is to determine the maximum tolerated dose to be used in future studies. These studies evaluate safety, tolerability, and dose-limiting toxicities in cohorts of patients receiving increasing doses of the test article. Antitumor efficacy is generally NOT a primary goal of phase I trials. In fact, response rates in phase I trials rarely exceed 10%.⁶⁴ In veterinary oncology, the phase I enrollee may have failed standard-of-care (SOC), no meaningful SOC exists, or the SOC is declined by or not economically feasible for the client. The type of patient enrolled in a veterinary oncology phase-I clinical trial is generally less heavily pretreated, with better performance status and potentially lower disease burden than are most human patients entering phase-I oncology trials, which could have implications for both the evaluation of efficacy and tolerability.

In the era of targeted agents, where there may not be a linear correlation between dose and efficacy, a different endpoint of phase I studies can be the biologically effective dose.⁶⁵ This is the dose that inhibits the intended target, which could be considerably lower than the conventional maximum tolerated dose. Assessing the biologically effective dose optimally requires sampling of either tumor tissue or surrogate tissue (eg, blood) to evaluate target modulation. The increased compliance and relative ease with which this can be performed in dogs vs human cancer patients, in part due to the fact that many other hospital procedures in these canine patients are also already being performed under anesthesia, is a distinct advantage of evaluation of targeted agents in tumor-bearing dogs, as evidenced by recent studies including canine phase I trials of the autophagy modulator hydroxychloroquine,⁶⁶ the Btk inhibitor ibrutinib,⁶⁷ the KIT tyrosine kinase inhibitor toceranib,⁶⁸ and the histone deacetylase inhibitor sodium valproate.⁶⁹

Phase II Trials (Early Antitumor Activity)

The purpose of a phase II trial is to identify clinical or biologic activity in well-defined patient populations (eg, tumors of a particular histology or tumors with a particular molecular target) and to inform the decision to embark on larger phase III trials. The traditional phase II trial is a single-arm, nonblinded

activity assessment of a novel therapy⁷⁰ using a fixed dose of test article established in phase I trials. Trial endpoints can include objective response rate, changes in imaging-based endpoints such as PET-CT glucose uptake, or changes in peripheral blood or tumor biomarkers. Again, the ease with which serial tumor sampling can be performed in dogs with cancer facilitates the validation of non- or minimally invasive (eg, imaging-based or peripheral blood, respectively) endpoints for use in future human or animal studies, as demonstrated in recent canine clinical trials.^{67,71}

Phase III Trials (Comparative/Confirmatory Trials)

In human oncology, phase III trials are large, ranging anywhere from 300 to 3000 patient, randomized, blinded controlled trials, with the goal of comparing a new drug or combination to treatment regarded as the standard of care.⁷² These are often multicenter studies to ensure more rapid case accrual. They are not common in veterinary oncology due to their size and expense, with the exception of several recent “registration” trials that have formed the basis for New Animal Drug Applications with the FDA Center for Veterinary Medicine.^{73–75}

Standardization and Accuracy in Evaluating Safety and Efficacy

One potential concern with regard to the comparability of human cancer clinical investigations and companion animal studies surrounds the rigor and standardization of efficacy and safety measurements in veterinary oncology. This concern has been largely mitigated over the past decade, with the publication of a series of consensus statements developed by the Veterinary Comparative Oncology Group, which standardizes response criteria for both solid tumors and lymphomas^{76,77} and standardizes reporting of severity and attribution of adverse events.⁷⁸ These criteria have been nearly universally accepted by the veterinary oncology community and have significantly improved the quality of safety and efficacy data reported in veterinary oncology clinical trials. Importantly, unlike laboratory animals, pet animals enrolled in clinical trials receive the same types of supportive care and symptomatic treatment as human patients, which again increases the translatability of results generated in these studies.

Table 3 Goals of Phase I–IV Clinical Trials

Clinical Trial Phase	Primary Goals	Secondary Goals
Phase I (Dose finding)	<ul style="list-style-type: none"> Determine MTD (or BED) Define DLT Describe other AEs 	<ul style="list-style-type: none"> PK/PD correlations Scheduling Preliminary efficacy
Phase II (activity/efficacy)	<ul style="list-style-type: none"> Determine activity/efficacy in defined populations Inform the decision to move to a phase III trial 	<ul style="list-style-type: none"> Estimate therapeutic index Expand AE data Expand PK/PD information Explore predictors of outcome
Phase III (comparative)	<ul style="list-style-type: none"> Compare efficacy of a new drug or combination to current “standard of care” 	<ul style="list-style-type: none"> Quality of life comparisons Comparative cost assessments
Phase IV (postmarketing)	<ul style="list-style-type: none"> Expanded AE and risk/benefit evaluation 	<ul style="list-style-type: none"> Evaluate special populations Explore additional indications

AE, adverse effect; BED, biologically effective dose; DLT, dose limiting toxicity; MTD, maximally tolerated dose; PK/PD, pharmacokinetic/pharmacodynamic.

Technical and Ethical Considerations for the Conduct of Research in Companion Animals vs Laboratory Animals

Institutions that utilize government funds to conduct animal research must adhere to the Public Health Service Policy (PHS Policy). The PHS Policy endorses the US Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training (IRAC Principles) and requires institutions to follow the Guide for the Care and Use of Laboratory Animals (the Guide) (<https://olaw.nih.gov/policies-laws/phs-policy.htm>). In the United States, any research conducted on covered species must comply with the Animal Welfare Act (AWA) (CFR, Title 9, 2013).

To conduct animal-based research, both the AWA and the Guide require institutions to establish and maintain an Institutional Animal Care and Use Committee (IACUC). The responsibilities of the IACUC are expressed in the AWA, the Guide, and in the Letter of Assurance the institution files with the NIH's Office of Laboratory Animal Welfare (<https://grants.nih.gov/grants/olaw/references/phspol.htm#Applicability>).

An effective IACUC works in collaboration with veterinary, research, and animal care staff to assist the institution in maintaining full compliance with all laws and regulations. IACUCs conduct reviews on the proposed animal research, including the impact of any procedures on animal well-being whether the animals are client or institution owned. IACUCs verify that animal research is performed with due consideration of the relevance to human or animal health, the advancement of knowledge, or the good of society, and that animal research is conducted in a manner that avoids or minimizes distress and pain.

The vast majority of research institutions operate animal-based research programs that exclusively involve animals housed in their facilities. For these institutions, the inclusion of client-owned animals in research projects presents unique ethical challenges that are not addressed in the AWA or by PHS policy. These unique considerations include identifying animals to enroll in studies, maintaining access to these animals, and ensuring ethical and legal matters are addressed such as obtaining informed consent and protecting animals and owners from conflict of interest issues.

IACUCs are responsible for the oversight of research conducted on animals. Importantly, the USDA has provided guidance that IACUC approval is not necessary for standard veterinary care of animals in the context of a veterinarian-client-patient relationship, nor does the USDA provide oversight to animals in the context of such a relationship.⁷⁹

The Ethical Principles and Guidelines for the Protection of Human Subjects of Research (NCPHS 1979; <https://www.hhs.gov/ohrp/regulations-and-policy/belmont-report/index.html>) have been used as the foundation to identify and address the ethical concerns associated with enrolling client-owned animals in research. The Ethical Principles and Guidelines are comprised of 3 main tenets: (1) respect (the client/owner is able to make informed choices), (2) beneficence (animal subjects should be protected and their well-being secured), and (3) justice (enrollment on study should be based on reasons directly related to the problem being studied). Per the Office of Humane Research Protections, application of these principles leads to consideration of informed consent, risk/benefit assessment, and the selection of research subjects.

Owners must be provided information that allows them to fully understand the research project in order to provide

informed consent. The owner should be made aware of the commitment being undertaken and the potential risks and benefits. Every effort should be made to deliver the information free from medical jargon, and it should be provided in an organized manner and in a situation that gives the client time to process the information and ask questions. The client's vulnerability should be taken into consideration, particularly if they are desperate to cure a pet's medical problem and if a pet owner is unable to afford conventional therapy.^{80,81} The potential conflict of interest arising from client/owner vulnerability becomes particularly important with regards to the enrollment of student- or staff-owned animals in clinical trials, a practice that is not uncommon at veterinary teaching hospitals. Specifically, extra precaution must be taken during the consent process to ensure that these individuals do not feel coerced into enrolling their pets into clinical studies, even if only as control animals. To minimize coercion, client informed consent documents should contain specific statements confirming that any owner decision regarding enrollment of their pet will have no impact on their academic standing or employment at the university.

The owners must be made aware of alternative options for treatment, including traditional standard therapies, institutionally defined secondary treatment protocols, and other investigational therapies. Owners should understand that the care their pet receives will not be diminished or harmed by declining to participate in a study. They should also understand how new information will be delivered to them, particularly if this information has the potential to affect consent, for example, if there are unanticipated side effects. Lastly, owners should understand how adverse events will be managed and specifically which party is financially responsible for the medical management of these events as well as the expectation for postmortem evaluation.^{80,81} Of important note, for the case of student- or staff-owned animals enrolled in clinical trials described above, additional steps must also be taken to prevent any conflict of interest regarding the medical management of these patients. To prevent any potential introduction of bias into the study, statements should be in place that guarantee that students or staff will not be involved in the medical management of their animal while on study.

Some veterinary institutions have adopted a board of review, termed a clinical review board (CRB), which functions similarly to an institutional review board and serves to evaluate the merit, feasibility, and compliance with ethical standards for clinical trials in client-owned animals. These boards should be in full communication with the IACUC. How individual institutions accomplish this communication can vary, but a possibility is to include members that serve on both committees.⁸²

The AVMA also has addressed how to manage studies using client-owned animals and published a policy entitled Establishment and Use of Veterinary Clinical Studies Committees (VCSC) (<https://www.avma.org/KB/Policies/Pages/Establishment-and-Use-of-Veterinary-Clinical-Studies-Committees.aspx>). According to this policy, the VCSC evaluates clinical research that conforms to general standards of care and then refers studies to the IACUC if patient management is affected by the study. The policy further states that the VCSC should ensure there is informed consent and that it should protect animals from conflict of interest issues.

To evaluate research involving client-owned animals, the current "best-practice" recommendation is to establish a CRB, which is charged with reviewing clinical studies and informed

consent.⁸² To ensure adequate expertise and communication between the CRB and IACUC, Baneux et al recommend at least one member of the CRB is a member of the IACUC and one member of the IACUC is a member of the CRB. Furthermore, it is recommended that someone with expertise in animal welfare be included on the IACUC, CRB, or both.

The point of entry of many client-owned animals onto research trials is at a veterinary teaching hospital. However, translational researchers may be located outside of academia, for example at a large private veterinary practice that does not have an IACUC or a CRB. A study conducted at a private practice might fall under the assurance of an institution with an IACUC. This is the case if the study is funded through a grant to the institution and the institution is responsible for compliance, including the informed consent process and its documentation, communication of adverse events with other sites involved in the study, and postapproval monitoring procedures. If there is no affiliation with an institution with an established IACUC or CRB, a contract research organization may be commissioned to meet compliance responsibilities.⁷⁴

NIH's NCI has posted on its website a 7-page list of research programs and researchers (<https://cancercenters.cancer.gov/Documents/researchprogramsreported2014-508c.pdf>). At the time this article was drafted, the list included 69 research programs, 5 of which were located on campuses with veterinary schools and included over 400 MDs and/or PhDs and one DVM.

The NIH and FDA encourage the maximization of data in research by fostering communication and collaboration (<http://www.ascopost.com/issues/august-25-2016/how-the-oncology-center-of-excellence-plans-to-foster-collaboration-among-researchers-to-advance-cancer-treatment/>). A stronger effort should be made to include visibility and connectivity of veterinary schools and client-owned animals in translational medicine.

Finally, it is critically important that after clinical trials are initiated in client-owned animals that postapproval monitoring and reporting continue to assure that animal welfare issues that may arise are addressed, the study protocol is adequately adhered to, and compilation and recording of data are uniform. The AVMA supports the care of animals in clinical studies be overseen by a Veterinary Clinical Studies Committee (<https://www.avma.org/KB/Policies/Pages/Establishment-and-Use-of-Veterinary-Clinical-Studies-Committees.aspx>). Conceivably, this committee could and in fact at many institutions does provide assistance with things such as drafting informed consent letters, documenting Good Clinical Practices, and assisting with patient recruitment.

Conclusion

Clinical trials in pet dogs with spontaneous cancer are important and underutilized translational models, owing to dogs' large size, relative outbreeding, and biological/physiological similarity to humans. Although yet to be fully defined, it is likely that the spontaneous development of these cancers, under the persistent selective pressure of an intact immune system that has coevolved with the tumor, more faithfully recapitulates the complex processes of metastasis and therapeutic resistance that remain major clinical hurdles in the treatment of human cancer patients. Canine tumor burdens are similar to humans, which may be important with regard to biological factors such as hypoxia and clonal variation and which also allows for serial imaging and tissue collection over time.^{10,83} This is due in part to the fact that these clinical

patients are routinely anesthetized for these procedures, mitigating owner concerns regarding patient discomfort. As such, clinical trials in companion animals with naturally occurring cancer have played and continue to play an important and informative role in human oncology.

The recent advances in molecular targeted therapies, and now immunotherapy, afford additional valuable opportunities to accelerate cancer drug development through this comparative approach. From further investigation into the suitability of the canine model via characterization of the molecular and immunologic landscapes of translationally relevant canine tumors, to evaluation of PD endpoints and biomarkers associated with drug response, the continued success of this approach, in part, will depend on the increasing incorporation of veterinary pathologists into comparative oncology research, trial design, and implementation. Furthermore, continued advancement of the field of comparative oncology will require increasing leadership, in the forms of intellectual input and innovation, from the veterinary pathology profession. Comparative oncology is a rapidly expanding field that will continue to be increasingly utilized for cancer drug development. Yet this discipline has predominantly lacked a truly unified scientific organization to provide oversight and recommendations for clinical trial and translational research conduct, likely resulting in certain missed opportunities due to the inherent pitfalls of individual and institutional-driven conventions for scientific approach.

In this regard, veterinary pathologists are uniquely situated in a position to come together as a profession and to interact with other members of the veterinary medical community to play a driving role in the establishment of standard operating procedures and best practice guidelines for critical components of comparative oncology trial conduct. Specifically, veterinary pathologists should take initiative in ensuring greater scientific rigor and standards for the continued histological and molecular characterization of spontaneous companion animal cancers while also advocating for, participating in, and overseeing the conduct of appropriately powered prospective studies aimed at validating pathological prognostic parameters for these tumors. In this age of precision medicine and the approval of digital pathology in human cancer diagnosis, veterinary pathologists could help to usher in this same technology to move the field of comparative oncology towards the establishment of a large, open-source database of molecular and pathological information on companion animal cancers. Overall, if implemented, these contributions will both lead to an increasing role of the pathologist in the clinical management of veterinary cancer patients while helping to maximize the insights into oncology drug development gained from translational investigations in animals with naturally occurring cancers.

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