HHS Public Access

Author manuscript

Annu Rev Anim Biosci. Author manuscript; available in PMC 2019 January 02.

Published in final edited form as:

Annu Rev Anim Biosci. 2016 ; 4: 199–222. doi:10.1146/annurev-animal-022114-110911.

Dogs as a Model for Cancer

Heather L. Gardner[1], **Joelle M. Fenger**[1], and **Cheryl A. London**[1,2]

[1]Department of Veterinary Clinical Sciences, The Ohio State University, Columbus, Ohio 43210; london.20@osu.edu

[2]Department of Veterinary Biosciences, College of Veterinary Medicine, The Ohio State University, Columbus, Ohio 43210; london.20@osu.edu

Abstract

Spontaneous cancers in client-owned dogs closely recapitulate their human counterparts with respect to clinical presentation, histological features, molecular profiles, and response and resistance to therapy, as well as the evolution of drug-resistant metastases. In several instances the incorporation of dogs with cancer into the preclinical development path of cancer therapeutics has influenced outcome by helping to establish pharmacokinetic/pharmacodynamics relationships, dose/regimen, expected clinical toxicities, and ultimately the potential for biologic activity. As our understanding regarding the molecular drivers of canine cancers has improved, unique opportunities have emerged to leverage this spontaneous model to better guide cancer drug development so that therapies likely to fail are eliminated earlier and therapies with true potential are optimized prior to human studies. Both pets and people benefit from this approach, as it provides dogs with access to cutting-edge cancer treatments and helps to insure that people are given treatments more likely to succeed.

Keywords

cancer; comparative oncology; clinical trials; dog; preclinical model

INTRODUCTION

The study of naturally occurring cancers in dogs provides a valuable perspective distinct from that generated with other animal models because dogs spontaneously develop cancers that share many characteristics with those found in their human counterparts. Cancers in pet dogs often recapitulate the biology and heterogeneity of human disease, including complex interactions between the immune system and tumor cells, significant heterogeneity, development of chemotherapy resistance, and metastasis resulting in patient death. Advances in the development of genome-integrated molecular reagents and commercially available high-throughput methodologies specific for dogs have enhanced our ability to more thoroughly interrogate canine cancers and characterize shared and novel targets for

DISCLOSURE STATEMENT

C.A.L. has served as consultant for Zoetis, Synta Pharmaceuticals, and Karyopharm Therapeutics. The remaining authors (H.L.G. and J.M.F.) are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

intervention. Importantly, specific biochemical pathways known to be drivers in human cancers are frequently observed in canine cancers, offering the opportunity to target those mechanisms in dogs and allow accurate preclinical assessment of novel therapeutics.

The fundamental similarities between cancer in dogs and people underscore the value of comparative and translational research for the benefit of both species. Clinical trials evaluating novel therapeutics in dogs with cancer provide dogs with state-of-the-art therapy at little or no cost and generate critical new data with direct applicability to subsequent human clinical studies. Consequently, multiple national collaborative initiatives, as well as biomedical industry entities, now leverage client-owned dogs with spontaneous tumors to address central questions regarding the biologic activity, adverse event profile, appropriate dose/regimen, and pharmacodynamic/pharmacokinetic (PK/PD) endpoints for novel cancer treatments with the ultimate goal of accelerating their application to human cancers.

NATURAL HISTORY OF CANCER IN DOGS

Cancer Incidence

Cancer is the most common cause of death in dogs, affecting approximately four million dogs per year. Several veterinary population-based cancer registries have provided information on risk factors as well as geographic and breed differences in the incidence of cancer. The earliest study, published in the 1960s, attempted to identify all tumors diagnosed in animals living in Contra Costa and Alameda counties over a three-year period (1). Subsequent epidemiologic studies in veterinary medicine have reported on the incidence of cancer in specific populations, with varied results (2–4). Canine cancer registries have provided important information regarding estimates of spontaneous tumor incidence in dogs; however, these studies are largely retrospective in nature and are restricted to a defined patient population (2, 5, 6). A retrospective necropsy study of 2,002 dogs reported 45% of dogs 10 years of age or older and 23% of dogs of all ages died of cancer, making it a leading cause of death in this species (5). The overall estimated incidence of malignant neoplasia in companion animals reportedly ranges from approximately 381 to 852 per 100,000 dogs (1, 2, 7). Data from the Animal Tumor Registry of Genoa estimated that the incidence of cancer in dogs ranged from 99.3 to 272.1 per 100,000 dogs (8). These data are comparable to the estimated cancer incidence in humans reported by the National Cancer Institute SEER program (http://seer.cancer.gov).

Disease Course of Common Tumors

Pet dogs share the same living environment as their caregivers and potentially serve as epidemiologic or etiologic sentinels for the changing patterns of cancer development observed in humans (9). In many cases, canine cancers are described in the same language as that of their human counterparts and can be classified according to histologic and/or clinical staging systems used in human cancers (e.g., National Cancer Institute Working Formulation, World Health Organization histopathological classification system) (10, 11). Although cancers in dogs share key features with those in humans, including biological behavior and responses to traditional regimens, such as surgery, chemotherapy, and radiation therapy (12), disease progression is typically much more rapid in dogs, permitting an

assessment of the impact of novel treatments in a timely manner (i.e., over 1–2 years rather than 5–10 years). Additionally, the incidence is higher for certain tumor types in dogs compared with people. For example, fewer than 1,000 people are diagnosed with osteosarcoma (OSA) each year, whereas OSA is at least 10 times more prevalent in dogs (13). This provides a significantly larger patient population in which to evaluate new treatment strategies and allows for rapid enrollment and completion of studies, particularly those performed in the setting of microscopic metastatic disease.

Evolution of Cancer Therapy in Veterinary Medicine

The treatment of cancer in veterinary medicine has coevolved with the treatment of cancer in people. The first report of chemotherapy use in veterinary medicine was published in 1946, describing the use of urethane to treat hematopoietic neoplasia in a dog (14). The incorporation of chemotherapy in veterinary medicine then closely paralleled its integration into human medicine, with the first combination chemotherapy protocol using chlorambucil and prednisone in dogs published in 1968 (15). Studies in dogs also had an impact on the clinical application of early cancer vaccines and bone marrow transplantation techniques (16–18). Treatment of dogs with OSA helped to develop novel limb-sparing surgical approaches, resulting in the optimization of limb-sparing techniques for children with this disease (13, 19). More recently, clinical trials in companion animals have been used to evaluate the safety and efficacy of new therapeutics and establish PK/PD endpoints. Conditional US Department of Agriculture (USDA) approval of the canine melanoma vaccine (ONCEPT®, Merial) was obtained in 2007, and toceranib phosphate (Palladia®, Zoetis), the first Federal Drug Administration-approved drug to treat canine cancer, was approved in 2009.

The changing paradigm of cancer treatment necessitated the establishment of an infrastructure to facilitate the inclusion of pet dogs with naturally occurring cancers in the drug-development path and to support continued comparative and translational research. The Comparative Oncology Trials Consortium (COTC) is a network of academic comparative oncology centers established in 2004 that performs controlled preclinical trials of diagnostic techniques and novel therapies in dogs with the primary goal of gathering critical new information to inform the design of subsequent human studies. The COTC Pharmacodynamic Core was implemented to ensure integration of PK/PD biological endpoints into these studies (20). Lastly, the Canine Comparative Oncology and Genomics Consortium has established a biospecimen repository as a resource to facilitate comparative genomics and the identification of valid tumor targets in canine cancers to aid in preclinical drug development.

DOG GENETICS: LINKAGE DISEQUILIBRIUM AND CANCER

Dog Breeding and Relatedness

The selective breeding of dogs for physical traits and specific functions dates back to the Industrial Revolution. As a result of population bottlenecks and continued breeding, there is a diverse array of dog breeds, some descending from a few founders, with documented increased risk for certain diseases (21). Numerous well-established breed predispositions for

specific cancers exist in veterinary medicine. For example, large-breed dogs are predisposed to developing appendicular OSA, whereas Scottish terriers are at increased risk for developing transitional cell carcinoma (TCC). Breed predilections for certain tumors support the notion that there is a shared core genetic predisposition and thus a shared common founder during breed development. Each breed is on the order of a hundred-fold genetically simpler than the full dog (or human) population. Thus, dogs are genetically extremely similar within breeds but are dramatically different across breeds. Importantly, the relative genetic simplicity of dog breeds and the canine model provides an opportunity to develop an understanding of somatic and germline contributions to diverse cancer traits.

Linkage Disequilibrium and Its Role in Identifying Disease-Related Genes

Interrogation of the genetic aberrations associated with naturally occurring dog populations provides information that uniquely contributes to our understanding of disease susceptibility in dogs and people (22). Linkage disequilibrium (LD) is on the order of a hundred-fold greater within dog breeds compared to that found within people, supporting the mapping of genetic traits in single breeds and among related breeds (23). Furthermore, the genetic and phenotypic homogeneity combined with high LD present within dog breeds allows for reduced sample size in canine genome-wide association studies (GWAS) compared with similar studies conducted in humans, further increasing the strength of dog models for mapping complex disease traits (22,23). Whereas LD within breeds is longer, LD across breeds is similar to that found in humans. This suggests that these traits are shared among breeds, while long LD correlates with breed segregation and is useful for broad genetic mapping (22).

Sequencing of the canine genome has further accelerated the usefulness of the dog model by identifying plausible candidate genes and single-nucleotide polymorphisms (SNPs) potentially useful for mapping breed-specific traits. Dogs are naturally susceptible to most cancers found in humans, and cross-species genomic studies often demonstrate involvement of the same genes and molecular pathways. Given that several dog breeds consistently show predilections for certain types of cancers, canine breed models provide a unique opportunity to map genetic variants associated with cancers. Furthermore, cancer involves complex germline-risk genetics and somatic mutations and is associated with broad types of environmental stimuli; thus, the relatively simplified genetics of dogs represents a significant advantage when studying germline cancer genetics. Several examples are discussed below.

Mast Cell Tumors in Golden Retrievers and Hyaluronidase Genes

Mast cell tumors (MCT) are the most common cutaneous tumor in dogs, and several breeds are predisposed to the development of MCT, including those of bulldog descent, retrievers, cocker spaniels, and shar-peis (11). Recently, a GWAS of canine MCT undertaken in two populations of golden retrievers compared the genomes of healthy golden retrievers with that of golden retrievers with MCT to identify germline risk factors associated with an increased risk of MCT (24). SNPs were identified on several chromosomes, and two chromosomal regions strongly associated with MCT harbor multiple hyaluronidase genes *(HYAL1, HYAL2,* and *HYAL3* on cfa20 and *HYAL4, SPAM1,* and *HYALP1* on cfa14), suggesting that turnover of hyaluronic acid plays an important role in the development of MCT.

Hyaluronidase normally degrades hyaluronic acid, an important part of the extracellular matrix that has been shown to restrict proliferation of cutaneous mast cells (25, 26). By stimulating anchorage-independent growth and proliferation, it is possible that hyaluronidase mutations could promote a more favorable tumor microenvironment, thereby contributing to tumor progression. The identification of germline risk factors in dogs with MCT may help identify at-risk populations and implicate variant genes and genetic networks important in normal and malignant mast cell biology with applicability to both humans and dogs.

Osteosarcoma in Greyhounds and Performance Genes

The identification of genomic alterations in human OSA that drive tumor initiation and development remains a challenge due to the high genetic instability and karyotypic complexity that are characteristic of this cancer (13, 27). Canine and human OSA exhibit overlapping transcriptional profiles, shared regions of genomic instability, and gene alterations, supporting the idea that these diseases are similar at the molecular level (13). Large- and giant-breed dogs, including Rottweilers, Irish wolfhounds, greyhounds, and Great Danes, exhibit a significantly increased risk for developing OSA, and evidence supports a breed-associated mode of inheritance (13, 27). The first GWAS for canine OSA susceptibility were conducted using samples from Rottweilers, Irish wolfhounds, and greyhounds. Numerous genome-wide inherited risk loci were identified, including prominent cancer genes *CDKN2A/B, AKT2,* and *BCL2.* Thirty-three inherited OSA loci that showed no overlap among the three breeds were implicated, consistent with the notion that a large part of the genome is fixed but can harbor mutations in disease-specific traits. Interestingly, these OSA-associated loci explained 57% of the phenotype variance in greyhounds and 55–85% of the phenotype variance when all breeds were considered. The top greyhound candidate locus was fine-mapped to a 6-Mb region on a chromosome 11 interval spanning *CDKN2A/B.* This locus was evaluated in eight additional dog breeds with high rates of OSA and was found to be highly rearranged in OSA tumors from multiple breeds (28). Furthermore, an enhancer screen in the human U2OS cell line narrowed the causative variant to a highly conserved single nucleotide predicted to lie within a PAX5 transcription factor binding site. PAX5 is implicated in the regulation of both B-cell and osteoblast differentiation and bone formation, suggesting a possible mechanism for the initiation or progression of OSA (29, 30). This work demonstrates the power of combining germline and somatic genetics to identify key factors important in canine OSA biology, which may help predict prognosis and identify relevant therapeutic targets in both humans and dogs (27).

Histiocytic Sarcoma in Bernese Mountain Dogs and CDKN2A/B Gene

Several breeds of dog are at increased risk of developing histiocytic sarcoma, including the Bernese mountain dog, flat-coated retriever, Rottweiler, and golden retriever (11). A pedigree analysis of 327 Bernese mountain dogs suggested that relatively few genes likely determine disease inheritance and could best be explained by an oligogenic model (31). Using molecular cytogenetics, researchers identified 31 recurrent copy number abberations (CNAs) present in the majority of both Bernese mountain dogs and flat-coated retrievers. Additionally, the six most common CNAs were deletions in chromosomal regions harboring

the tumor suppressor genes *CDKN2A/B, RB1,* and *PTEN,* with loss of *CDKN2A/B* seen in 60.7% of Bernese mountain dogs (32).

More recently, a GWAS of histiocytic sarcoma identified cancer-associated loci in Bernese mountain dogs from the United States and Europe (33). When dogs from both groups were considered together, there was genome-wide significance on the CFA 11 locus. Fine mapping of the CFA 11 locus demonstrated that all dogs with the case-associated allele had an identical three-SNP haplotype found on at least one chromosome in 96% of all dogs with histiocytic sarcoma. These three SNPs overlap the gene *MTAP* and the tumor suppressor gene *CDKN2A/B,* providing further support for the notion that *CDKN2* dysregulation likely plays a role in the pathogenesis of this disease (33).

Squamous Cell Carcinoma in Standard Poodles and the KITLG Locus

Squamous cell carcinoma (SCC) frequently arises in the digit, representing 47.4% of all malignant digital tumors in dogs (11). Several breeds have an increased risk of digital SCC, including the giant schnauzer, Gordon setter, briard, Kerry blue terrier, and standard poodle (34). A GWAS of black standard poodles identified a region on chromosome 15 that significantly correlated with digital SCC development in this breed, with 90% of cases containing the risk-associated allele. Additional fine-mapping and haplotype analysis resolved this region to the *KITLG* (KIT ligand/stem cell factor) locus, and it was subsequently determined that although light-colored dogs carry the copy number variation, they have a significantly lower risk of developing SCC. The only difference identified between light- and dark-colored standard poodles in this study was a mutation in the *MC1R* locus, suggesting that an interaction between the *KITLG* and *MC1R* loci is required for oncogenesis (34). Both *KITLG* and *MC1R* are important for skin and coat color, and *KITLG* has been associated with human testicular cancer in two separate studies (35, 36). By studying traits within and between dog breeds, multigene interactions important for the pathogenesis of SCC in both dogs and people were identified.

BIOLOGY OF CANCERS IN DOGS AND RELATEDNESS TO HUMAN DISEASE

Osteosarcoma

As previously discussed, although OSA is the most common primary bone tumor in both people and dogs, it is significantly more prevalent in dogs, with a reported incidence of 13.9/100,000 in dogs in contrast to 1.02/100,000 in people (13). OSA commonly occurs in older dogs (median age 7 years); however, a bimodal distribution is present with a small peak in young dogs (average age 1 year). This is in contrast to human OSA, which is more common in adolescence (10- to 14-year-old age group) (13). Amputation and adjuvant chemotherapy improve median survival times to 8–12 months from the 3–4 months typically achieved with amputation alone; however, 90% of dogs are euthanized within 2 years of diagnosis (11). Similarly, the prognosis for OSA in children is guarded; the overall 5-year survival rate is 67% in the nonmetastatic disease setting and 10–30% if metastases are found at initial diagnosis (13).

Consistent with the notion that canine OSA is a relevant, spontaneous, large-animal model to study pediatric OSA, gene and signaling-pathway alterations fundamental to disease pathogenesis are highly conserved in the human and canine disease. Comparative genomic analyses have characterized shared abnormalities and have identified novel molecular drivers that may be relevant targets for therapeutic intervention. Dysregulation of specific candidate genes implicated in the etiopathogenesis of OSA are found in both species, including mutations in the tumor suppressor genes *p53, RB1,* and *PTEN* and alterations of the oncogenes *MYC* and *MET,* among others (13). Cluster analysis of orthologous gene-expression signatures does not discriminate between canine and human OSA on the basis of species, suggesting that cancers from each species are indistinguishable by gene-expression analysis. Furthermore, the reduced genetic heterozygosity in breeds of dogs can help identify candidate genes and/or molecular subtypes in OSA (27, 37). For example, *IL-8* and *SCL1A3* were found to be overexpressed in canine OSA. Subsequently, expression of these genes was found to be associated with an aggressive clinical course and poor outcome in human OSA (27).

p53 is a tumor suppressor that functions primarily as a transcription factor for numerous genes involved in DNA repair, proliferation, and apoptosis. There is strong homology and similar incidence of mutations in the highly conserved coding regions of the *p53* gene in dog and human OSA (38). p53 is often overexpressed in canine primary OSA samples, with the frequency of reported mutations ranging from 23% to47% (13, 39–41). Although a similar incidence (15–30%) of *p53* mutations is found in human OSA, the nature of mutations differs, with point mutations more common in dogs and large alterations more common in humans (39, 42, 43).

Rb is another tumor suppressor protein for which loss of function is associated with the risk of OSA development in people and is implicated in the initiation and/or progression of OSA in both species (13, 44). Copy number loss of the *RB1* gene was detected in 29% of canine OSA tumor samples, corresponding to reduced or absent Rb protein expression in 8 of 13 (61.5%) samples analyzed (45). In support of these findings, genome-wide comparative genomic hybridization (CGH) profiles in canine OSA showed *RB1* loss in 36% of samples tested (28). These results are consistent with the reported 30–75% incidence of *RB1* genomic alterations in human OSA, supporting the notion that Rb dysregulation plays an important role in the pathogenesis of this disease in both species (46, 47).

Signal transducer and activator of transcription 3 (STAT3) is a transcription factor that mediates signal transduction to the nucleus in response to cytokine and growth factor receptor binding. STAT3 regulates genes involved in cell proliferation, apoptosis, angiogenesis, and immune responses (48). Constitutive activation of STAT3 is found in multiple tumors, including a subset of human and canine OSA tumors and cell lines (48–50). Furthermore, expression of high intratumoral phosphorylated STAT3 levels has been associated with a poorer prognosis in human OSA (51). Downregulation of phosphorylated STAT3 induces cell-growth arrest and apoptosis in canine and human OSA cell lines, supporting its potential as a target for therapeutic intervention (49, 51). Efforts are ongoing to develop small molecule STAT3 inhibitors, and several have been shown to have biologic activity in OSA tumor cell lines and xenografts, including FLLL32, LLL12, and Ly5 (52–

56). The current inclusion of dogs with spontaneously occurring OSA in the development and testing of therapeutics targeting STAT3 biology will likely provide important new information with direct relevance to future testing in people. Together these data suggest that the integration of dogs with OSA into comparative and translational cancer research has the potential to identify shared and novel therapeutic targets for intervention, lending additional insight into the biology of OSA and ultimately advancing the care of children affected by this disease.

Lymphoma

The biologic behavior of non-Hodgkin lymphoma (NHL) is similar between dogs and people, including response to standard chemotherapy and drug resistance. The incidence of NHL is reportedly between 15.5 and 29.9 per 100,000 in people and 15–30 per 100,000 in dogs; however, more recent studies indicate that the incidence of canine lymphoma may be higher (57, 58). B- cell lymphoma is more prevalent than T-cell lymphoma in both species, and diffuse large B-cell lymphoma (DLBCL) is the most common subtype diagnosed (6, 58). Breeds at increased risk of developing lymphoma include the bull mastiff, bulldog, boxer, Bernese mountain dog, Scottish terrier, and Gordon setter, among others (11). For example, a higher rate of T-cell lymphoma has been reported in the Irish wolfhound (6). Similarly, a high percentage of boxers (85%) develop T-cell disease, the majority of which are subclassified immunophenotypically as $CD3^+$ $CD4^+$ (59, 60). Taken together, these breed-specific distributions support the presence of heritable risk factors for lymphoma (6).

As in people, CHOP-based chemotherapy (vincristine, cyclophosphamide, prednisone, and doxorubicin) is typically used to treat dogs with lymphoma, with median survival times of 10–14 months and 6–9 months reported for dogs with B-cell and T-cell disease, respectively (11). The outcome for people with B-cell lymphoma is also better compared with T-cell lymphoma (61). Given the similarities in lymphoma between dogs and people, as well as the increased prevalence in specific breeds, dogs are often used as a model to study the biology of disease and the application of novel therapies (6).

Genetic conservation between hematologic cancers in dogs and people has been demonstrated in several studies. A recurrent translocation in canine B-cell lymphoma was identified in which *MYC* is placed under the control of the immunoglobulin heavy-chain promoter, leading to constitutive expression of Myc. *MYC* is dysregulated in human Burkitt's lymphoma, and this rearrangement is homologous to human t(8;14), demonstrating that cytogenetic abnormalities are conserved between dogs and people (62). Array comparative genomic hybridization (aCGH) has also been used to compare copy number imbalances between canine and human lymphoma. Cross-species aCGH analysis permits increased resolution in important areas of genomic gains/losses that would otherwise be impossible using a single-species genetic approach. Shared copy number aberrations, such as gains of *MYC,* were detected in canine and human lymphoma (63). Additionally, aCGH was used to identify copy number aberrations in 12 dogs with DLBCL that were associated with outcome. Fourteen recurrent copy number aberrations were identified along chromosomes 13 and 31 in regions containing several well-established oncogenic drivers, including *MYC, KIT,* and *PDGFRA* (64).

DLBCL is a heterogeneous disease in people, which is reflected by its classification into histological and molecular subtypes. In people, both gene expression profiling (GEP) and immunohistochemical algorithms are used to predict outcome (65). DLBCL is separated into two prognostic subcategories based upon well-defined genetic signatures: activated B-cell (ABC) and germinal center B-cell (GCB) DLBCL (65). Although the immunohistochemical algorithms used in human medicine do not directly translate to veterinary medicine, the availability of commercial GEP platforms has accelerated the usefulness of this technique in dogs. The first reported use of GEP in canine lymphoma grouped cases based on their cytomorphology and immunophenotypic properties, including low-grade T-cell, high-grade T-cell, and B-cell lymphoma (66). More recently, GEP studies identified genetic signatures that separated canine DLBCL into categories reminiscent of the ABC and GCB molecular subtypes used in people (67). In the human clinical oncology setting, ABC-DLBCL and GCB-DLBCL can also be molecularly subclassified based on the status of ongoing versus completed immunoglobulin heavy chain gene hypermutation. Immunoglobulin somatic hypermutation is an adaptive measure that is essential to permit immunoglobulin mutagenesis and maturation; however, in the setting of massive clonal expansion, germinal center B cells are prone to malignant transformation and subsequent progression to GCB-DLBCL. Ongoing somatic hypermutation status is more commonly observed in GCB-DLBCL, and this is associated with a more favorable prognosis. In contrast, ABC lymphomas arise from cells that have completed somatic hypermutation and therefore contain static immunoglobulin heavy chain variable region sequences. The absence of ongoing somatic hypermutation and its association with the ABC-DLBCL subtype in people reinforce the concept that malignant lymphoid cells develop at discrete stages of normal lymphocyte maturation and that they retain the genetic program of those normal cells (68). Immunoglobulin heavy chain mutation status in canine DLBCL also correlates with prognosis, and similar to human DLBCL, canine lymphomas harboring a static somatic hypermutation phenotype have a significantly shorter progression-free survival (67).

Canine DLBCL demonstrates significant overlap with human DLBCL, including altered FMS-like tyrosine kinase 3 (FLT3), nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), and B-cell receptor pathway signaling, providing further support for its utility as a model. For example, the NF-κB pathway is commonly dysregulated in human DLBCL. Comparative GEP in canine and human DLBCL demonstrated activation of the NF-κB/p65 canonical pathway (69). Increased expression of the NF-κB pathway genes in dogs with ABC-like DLBCL has been demonstrated, mirroring human ABC-DLBCL (67). Phase-1 studies have been conducted in dogs with DLBCL to evaluate the biological activity of inhibitors that block NF-κB activation (70, 71). These studies demonstrated the safety of these inhibitors and showed that constitutive NF-kB signaling could be effectively downregulated in a subset of dogs with ABC-like DLBCL, resulting in decreased malignant B-cell proliferation (70, 71).

More recently, inactivating mutations in *TRAF3* were first identified in canine DLBCL and then in a small number of human DLBCL samples (72). The *TRAF3* gene encodes a negative regulator of the noncanonical NF-κB pathway and was mutated in 44% of the canine samples, resulting in downregulation of gene expression. Loss of *TRAF3* was

subsequently identified in approximately 9% of human DLBCLs, supporting further studies evaluating the role of *TRAF3* in the pathogenesis of canine and human DLBCL.

Leukemias

Lymphocytic and myelogenous leukemias are a heterogeneous group of hematopoietic neoplasms distinguished by their individual clinical and biologic features and genetic aberrations, including gene mutations, chromosomal translocations, and altered activity of signaling proteins. The overall incidence of all types of leukemia in people is 9.7 per 100,000 cases (57). Although the true incidence of leukemia in dogs is unknown, two retrospective studies indicate that lymphocytic leukemia is more commonly diagnosed compared with myelogenous forms of leukemia, which are rare in dogs (73, 74). Treatment in people is dependent on immunophenotypic features, cytologic characteristics, and molecular subtype and can include bone marrow or stem cell transplant, chemotherapy, and/or targeted therapies.

Human chronic lymphocytic leukemia (CLL) frequently arises from a clonal proliferation of circulating $CD45^+$ B cells and represents the most common form of adult leukemia in people, whereas $CD8^+$ T-cell CLL is far more common in dogs (73, 75). In dogs, CLL is considered an indolent disease, and treatment typically involves the alkylating agent chlorambucil (11). Despite the difference in immunophenotype, conserved genomic alterations involving the *RB1* locus have been identified in canine and human CLL. Deletion or loss of heterozygosity of chromosome 13q14 is present in approximately 68% of human CLL patients, and this region contains the *RB1* gene locus (76). Asimilar deletion was identified in canine CLL at CFA22, which is homologous to the HSA 13q14 deletion present in human CLL (62). As in people, the CFA 22 deletion included the *RB1* locus, resulting in reduced or absent Rb protein expression in CLL samples from dogs. Thus, despite differences in the prevalence of B-cell versus T-cell CLL immunophenotypes found in dogs and people, the presence of comparable genetic aberrations suggests that these diseases may be similar on a molecular level.

The Philadelphia chromosome refers to a chromosomal rearrangement present in 95% of human chronic myelogenous leukemia (CML) cases (77). A reciprocal translocation [t(9;22)] between the *ABL* locus and the breakpoint cluster region (BCR) places the ABL1 gene under the control of the BCR promoter, producing a constitutively active cytoplasmic tyrosine kinase fusion protein, BCR-ABL (77). The use of imatinib (GLEEVEC®), which inhibits the BCR-ABL fusion protein, significantly increases survival times for people with CML by inhibiting cellular growth and inducing apoptosis (77). An equivalent translocation was identified in canine CML cells, termed the Raleigh chromosome (62). Subsequently, two case reports have identified *BCR-ABL* translocations in a dog with chronic myelomonocytic leukemia and acute myeloblastic leukemia (AML) (78, 79).

Acute lymphocytic leukemia (ALL) and AML are aggressive diseases in dogs, with poor survival times despite multiagent chemotherapy treatment (11). In people, many acute leukemias overexpress FLT3 and are associated with a poor prognosis (80). *FLT3* juxtamembrane internal tandem duplications (ITDs) or point mutations are more commonly seen in human AML as compared with ALL, representing approximately 25–30% and 1% of

cases, respectively (80). Interestingly, in one study *FLT3* ITDs were identified in three dogs (8%) with ALL (81). Corroborating these data, the presence of ITD mutations in spontaneous canine ALL was shown to result in activation of downstream JAK/STAT and MAP kinase signaling pathways, providing further evidence to support the notion that ALL and FLT3 biology are conserved between dogs and humans (82).

Transitional Cell Carcinoma

TCC in the urinary bladder of dogs shares many clinical, histological, and biological similarities with invasive high-grade TCC in people. Whereas high-grade, infiltrative TCC represents the most common histopathological diagnosis in dogs, the majority of bladder cancers in humans are classified as superficial low-grade TCC (83). Canine TCC is usually located in the trigone region of the bladder, containing the ureteral orifices and internal urethral orifice, whereas the distribution within the human bladder is more variable. In addition, a male predisposition is reported in people, whereas in veterinary medicine, female dogs have a higher incidence of TCC. Similar responses to treatment, frequency, and location of metastatic disease are observed in dogs and people with high-grade TCC (83). Necropsy studies have shown that regional metastasis to lymph nodes and distant metastases to lungs, bone, and other organs in invasive, high-grade TCC approaches 50% in both species (11, 83). Surgery (complete cystectomy) and chemotherapy, with or without radiation therapy, are standard therapeutic options for the treatment of high-grade TCC in people. Total cystectomy and trigonal resection have been described in dogs but are not routinely performed due to the morbidity associated with this procedure. Partial cystectomy in dogs can be an effective treatment modality in canine TCC; however, the majority of canine urothelial tumors are located in the trigone region and frequently involve the urethra and/or prostate, limiting the utility of this surgical option (11, 84).

Cyclooxygenase (COX)-2 overexpression has been documented in both canine and human invasive TCC and carcinoma in situ (83). The initial clinical evaluation of COX inhibitors in TCC was completed in dogs prior to the conduct of similar studies in people (85, 86). In a phase-2 clinical trial of piroxicam (a COX-1/COX-2 inhibitor) administered to 34 dogs with TCC, a 17% objective response rate [complete response (CR) plus partial response (PR)] was reported with an additional 18 dogs experiencing stable disease, for an overall clinical benefit (CR + PR + stable disease) of 70% (87). The results of this study prompted subsequent trials evaluating COX inhibitor therapy in people with carcinoma in situ. The antitumor effects of COX inhibitors in TCC are largely due to induction of apoptosis, and recent studies have provided evidence that piroxicam is associated with induction of apoptosis and reduction of tumor volume in dogs (86). Importantly, similar biologic effects have been observed in people with invasive TCC following treatment with other COX inhibitors (88).

Additional shared cellular and molecular features have been evaluated in canine and human TCC, including telomerase activity and altered expression/activation of several proteins (survivin, androgen receptor, urine basic fibroblast growth factor), among others (83). Interestingly, over 80% of canine invasive TCC tumors carry an activating mutation in *BRAF* homologous to the activating BRAF(V600E) mutation typically found in human

malignant melanoma and a small proportion of thyroid carcinoma and colon carcinoma patients (89). *BRAF* mutations are found in approximately 8% of all human cancers but are rare in human urothelial tumors (90). Despite this, given the high prevalence of mutation in canine TCC, this tumor may represent an interesting model of BRAF dysfunction that can be used to study the activity of novel small molecule inhibitors. These findings highlight the importance of looking beyond the histopathologic features of tumors and interrogating the genome to further our understanding of the molecular events driving cancer in humans and dogs.

Soft Tissue Sarcoma

Soft tissue sarcomas (STS) are a heterogeneous group of mesenchymal tumors subclassified based on biologic behavior, histologic appearance, and recurrent chromosomal aberrations. The overall incidence in people is 1% of all diagnosed cancers (91), whereas the reported annual incidence in dogs is 35 per 100,000 (11). In both dogs and people, local control is crucial for successful treatment, generally involving surgery with or without adjuvant radiation therapy (11, 92). The efficacy of chemotherapy for many canine and human STS in the gross or microscopic disease setting is generally poor. Ewing's sarcoma is an exception to this in human medicine, where the addition of multiagent chemotherapy to local therapy favorably impacts outcome (93).

The specific characterization of genetic aberrations has helped to further classify STS and identify factors associated with prognosis, as well as novel targets for therapeutic intervention. Recurrent chromosomal abnormalities have been identified in STS in people and, more recently, in the Labrador retriever (94, 95). Abnormalities in chromosomes 11 and 30 were found in primary cell cultures taken from two Labrador retrievers with anaplastic fibrosarcoma (94). These observations were extended in a subsequent study, in which complex chromosomal rearrangements on chromosome 11 in a region containing two tumor suppressor genes *(CDKN2A/CDKN2B)* were identified (96). Although this study did not provide prognostic information, loss of *CDKN2A/CDKN2B* in people with STS has been associated with a worse prognosis (97).

Similarly, genetic aberrations in the proto-oncogene *KIT* have been used to classify molecular subtypes of gastrointestinal stromal tumors (GIST) (11, 98). In people, GIST are driven primarily by activating mutations in *KIT,* with approximately 80% of tumors possessing activating mutations in exon 11 of this gene (98, 99). *KIT* mutations involving mutations in exon 11 have also been reported in 35.5% of canine GIST (100). Although mutations in *PDGFRA* have been reported in 5–10% of human GIST cases, these have not been identified in canine GIST (99–101). The presence of *KIT* mutations in human GIST led to the use of the small molecule inhibitor imatinib (GLEEVEC®) for inoperable or metastatic GIST, with objective response rates in affected patients exceeding 60% (101).

Mammary Cancer

Mammary tumors are the most common neoplasm identified in female dogs, accounting for up to 70% of tumors in European registries (8). In dogs, risk factors for development of mammary tumors include breed, age, hormonal exposure, and possibly obesity (11). In

veterinary medicine, the most consistently reported prognostic factors include clinical stage and tumor grade (11). A recent multivariate survival study assessed the value of several immunohistochemical markers and identified stromal cell MMP-9 expression and tumor cell Ki-67 expression as independent prognostic factors in canine mammary tumors (102).

In people, mammary cancer (i.e., breast cancer) is classified based on the presence or absence of specific molecular markers (103). The estrogen receptor (ER)-positive subtypes are Luminal A and Luminal B, whereas the ER-negative subtypes are HER2-overexpressing, normal breast-like, and basal-like subtypes (103, 104). The basal-like subtype is characterized by the lack of estrogen receptor, progesterone receptor, and HER2 expression. More recently, a claudin-low subtype has been characterized (105). The various subtypes not only are prognostic but guide subsequent treatment recommendations. Breast cancer in people is typically now further characterized by gene expression profiles and subclassification into molecular subtypes, and these are used to guide tumor-specific therapy that has significantly improved long-term outcomes.

To date, no studies have been performed in veterinary medicine to establish a molecular classification scheme for canine mammary tumors. Instead, most studies have focused on identifying risk-associated genetic aberrations and gene signatures to help delineate the molecular pathways associated with mammary tumors. For example, a candidate gene approach was used to evaluate genes implicated in human breast cancer in the English springer spaniel, a breed at increased risk of developing mammary tumors (106). This study demonstrated that germline mutations in *BRCA1* and *BRCA2* are significantly associated with mammary cancer in that breed (106). More recently, comparative pathway expression analysis of orthologous dog/human genes was performed in mammary tumor samples, demonstrating that gene signatures and cancer-related pathways were similar in both canine and human mammary tumor samples (107). Another study demonstrated the utility of GEP in canine mammary tumors and identified 17 genes differentially expressed in benign and malignant mammary tumors (108). Three genes *(BMP2, LTBP4, DERL1)* were defined that, when used in combination, correctly classified each tumor as either benign or malignant (108).

The identification of molecular drivers in the pathogenesis of human mammary cancer led to the successful development of targeted molecular therapies that have significantly improved patient survival. For example, epidermal growth factor 2 *(ERBB2, HER2, c-erbB-2, neu)* is a proto-oncogene overexpressed in 25% of human breast cancers and is associated with a poor prognosis (109). *HER2* expression is now used to identify those patients who will respond to trastuzumab (Herceptin®), a monoclonal antibody targeting HER2. In canine mammary carcinomas, loss of HER2 expression has been associated with a poor prognosis in conjunction with ER negative status and positivity of basal cell markers (P-cadherin, p63, cytokeratin 5) (110). HER2 protein overexpression is reportedly present in 20–29.7% of canine malignant mammary tumors, mirroring the incidence ofHER2 overexpression seen in human breast cancers (111, 112).

Melanoma

Both humans and dogs are affected by malignant melanoma, although the clinical presentation of disease tends to be different. In people, cutaneous melanoma is the most common presenting condition, and tumor development is linked to sun exposure. Nearly all cases of cutaneous melanoma in people are malignant, and molecular characterization has shown that an activating mutation in *BRAF* is the primary driver in at least 60% of affected patients. In dogs, cutaneous melanoma is typically benign, whereas oral melanoma (the most common form of the disease) is highly aggressive, associated with metastasis in at least 90% of affected dogs (11). This is in contrast to the case in people, where oral melanoma accounts for only 0.5% of all cancers observed in this location. However, the clinical course of oral (also known as mucosal) melanoma in people is equally aggressive with frequent metastasis. Resistance to chemotherapy is a feature of oral melanoma in both species (113).

Activating mutations in *KIT* have been identified in human malignant melanoma, with the largest subset of RTT-mutant-positive tumors representing mucosal melanomas (16%) (114). KIT expression is variable in canine melanoma, and activating mutations in *KIT* have not been confirmed. Furthermore, the role of wild-type or mutant KIT signaling and its contribution to melanoma tumorigenesis have not been characterized in canine malignant melanoma (115).

As previously mentioned, approximately 60% of cutaneous melanomas in people contain activating *BRAF* mutations, with the majority of tumors harboring V600E mutations (90, 116).In contrast, activating *BRAF* mutations have not been identified in canine or human mucosal melanomas (117–119), although MAPK and PI3K/AKT pathway dysregulation has been found in mucosal melanomas derived from both species (117). A possible contributing factor to this activation is *NRAS,* a GTP-binding protein that stimulates both MAPK and PI3K/AKT signaling pathways. *NRAS* mutations are typically present in cutaneous sun-induced melanomas in people (120). In contrast, *NRAS* is mutated in less than 30% of human mucosal melanoma, supporting the notion that melanomas arising from different sites exhibit distinct mechanisms of molecular transformation. Consistent with this idea, activating *NRAS* oncogene mutations are rare in canine oral malignant melanoma (117, 121). Together, these data suggest that the biology of canine melanoma shares more similarities to that of human melanoma arising from non-sun-exposed sites and may therefore serve as a good model to study potential novel therapies to treat this aggressive disease.

CLINICAL TRIALS IN DOGS WITH CANCER

Liposome Encapsulated Muramyl Tripeptide

Liposome encapsulated muramyl tripeptide phosphatidyl ethanolamine (L-MTP-PE; mifamurtide) is a lipophilic derivative of muramyl dipeptide, a synthetic analog of a mycobacterium cell wall component, which is incorporated into liposomes. L-MTP-PE exerts its anticancer effects by stimulating monocytes to kill tumor cells in vitro, and it induces pulmonary macrophages to kill tumor cells in murine xenograft models. A double-blind placebo-controlled clinical trial of L-MTP-PE in 27 dogs without evidence of gross

metastatic disease following amputation demonstrated a significant improvement in survival time over placebo alone (222 days versus 77 days) (122). Additionally, dogs with OSA that underwent amputation and received both cisplatin chemotherapy and L-MTP-PE treatment experienced significant improvements in overall survival (123). The results of these studies formed the basis for phase-2 and −3 clinical trials of L-MTP-PE in children with OSA. Although no difference in progression-free survival time was detected in the phase-3 study, the addition of L-MTP-PE to standard chemotherapy significantly improved overall survival (124, 125). This resulted in its approval for newly diagnosed nonmetastatic OSA in conjunction with chemotherapy by the European Medicines Agency in 2008, although it remains an investigational drug in the United States.

Toceranib/Sunitinib

Toceranib phosphate (Palladia®, Zoetis) is an orally bioavailable, multitargeted small molecule inhibitor that blocks several receptor tyrosine kinases, including VEGFR2, PDGFR, KIT, and FLT3, among others (126–129). A phase-1 study of toceranib in dogs with a variety of spontaneous tumors demonstrated an objective response rate of 28%, with an overall clinical benefit of 54%. The highest response rates were noted in dogs with MCT that harbored activating *KIT* mutations. Work with toceranib in dogs also established PK/PD endpoints, providing evidence of target modulation and information on drug levels, exposure duration, and the expected adverse event profile. Together these studies supported subsequent work with the very closely related small molecule inhibitor sunitinib in people (127). Both drugs were codeveloped by Sugen, Inc., with sunitinib ultimately chosen as the human clinical lead. Importantly, clinical trials of toceranib in dogs were completed prior to the phase-1 study of sunitinib in people, providing guidance regarding PK/PD relationships, anticipated clinical adverse events, and expected activity in KIT-driven malignancies such as GIST (130, 131). Sunitinib received FDA approval in January 2006 after clinical trials demonstrated efficacy against advanced renal cell carcinoma and imatinib-resistant GIST (130, 131).

Ibrutinib

Ibrutinib (PCI-32765; Imbruvica®) is an irreversible small molecule Bruton’s tyrosine kinase (BTK) inhibitor. BTK is recruited early in the BCR signaling cascade and contributes to the pathogenesis of several hematopoietic malignancies, including B-cell CLL, DLBCL, follicular lymphoma, and mantle cell lymphoma. Initial evaluation in hematopoietic cells and murine xenograft models demonstrated selective inhibition of B-cell signaling and BTK occupancy (132). Furthermore, ibrutinib demonstrated selective toxicity to ABC-DLBCL cell lines dependent on chronic active BCR signaling (133). Prior to human clinical trials, ibrutinib was studied in dogs with B-cell lymphoma, validating the use of a novel assay to demonstrate receptor (BTK) occupancy, biologic activity in a relevant large animal model of disease, and an acceptable adverse event profile (132). These findings provided support for the continued development of ibrutinib for B-cell malignancies in people, and a phase-1 study in relapsed and/or refractory B-cell malignancies confirmed both safety and efficacy (134). Subsequent clinical work demonstrated high response rates in B-cell CLL and mantle cell lymphoma (71% and 68% ORR, respectively) (135, 136), ultimately resulting in FDA approval of ibrutinib in 2014.

Melanoma Xenogeneic Vaccine

A variety of immunotherapeutic strategies have been explored for the treatment of human and canine malignant melanoma. Tyrosinase is a melanosomal protein integral for melanin synthesis. Preclinical murine xenograft models demonstrated that xenogeneic DNA vaccines with genes encoding melanosomal differentiation antigens can induce specific antibody and cytotoxic T-cell responses against syngeneic tumor cells (137). These studies provided the impetus for the development of a xenogeneic tyrosinase DNA vaccine for the treatment of canine malignant melanoma. Clinical trials of a xenogeneic plasmid DNA encoding human tyrosinase in dogs with oral malignant melanoma demonstrated the safety of the vaccine approach and provided some hints of potential efficacy with respect to long-term disease control (138). Additionally, induction of anticanine and antihuman tyrosinase-specific antibody responses was documented, coinciding with observed clinical responses (139). The xenogeneic human tyrosinase DNA vaccine (ONCEPT^{tm} Canine Melanoma Vaccine, Merial) received USDA approval in 2007, becoming the first and only USDA-approved therapeutic vaccine for the treatment of cancer in either dogs or humans. This body of work supported subsequent human clinical trials using a xenogeneic tyrosinase DNA vaccine for malignant melanoma.

Verdinexor/Selinexor

Exportin-1 (XPO1, CRM1) is a nuclear export protein responsible for exchanging proteins from the nucleus to the cytoplasm (140). XPO1 is commonly upregulated in spontaneous hematologic and solid tumors, and this correlates with a poor prognosis, indicating that changes in nuclear- cytoplasmic trafficking resulting in abnormal cellular compartmentalization of key proteins may contribute to tumorigenesis and potentially resistance to therapy (140, 141). Selinexor (KPT-330) and verdinexor (KPT-335) are orally bioavailable small molecule selective inhibitors of nuclear export that reversibly block XPO1 function. Initial studies evaluating the biologic activity of verdinexor were performed in dogs with spontaneous malignancies (142). A phase-1 study of verdinexor was performed in dogs with lymphoma, MCT, and metastatic OSA. Clinical benefit was observed primarily in dogs with lymphoma (13 of 20), with dose-limiting toxicities related to the gastrointestinal tract (anorexia and weight loss) that were alleviated with supportive care and dose modifications. Importantly, data from these canine studies were included in the Investigational New Drug (IND) application for selinexor. Furthermore, the clinical toxicities and responses seen in dogs were highly predictive of those observed in people treated with selinexor. A phase-2 clinical trial of verdinexor completed in dogs with T- and B-cell lymphoma confirmed findings from the initial study; the overall response rate was 37% (20/54 dogs), with 20 dogs remaining in the study for longer than 2 months.

STA-1474/Ganetespib

Heat shock protein 90 (HSP90) is a conserved molecular chaperone that facilitates maturation of client proteins to a biologically active conformation. Many of the HSP90 client proteins are oncoproteins, notably HER2/neu, EGFR, AKT, KIT, and MET, among others (143). STA-1474 is the water-soluble prodrug of STA-9090 (ganetespib), which binds the ATP-binding domain at the N terminus of HSP90 and induces degradation of multiple

oncogenic HSP90 client proteins (143). Based on promising in vitro studies and evaluations in mouse xenograft models demonstrating activity of STA-1474 in canine OSA and MCT cells lines and a murine OSA xenograft model (144), a phase-1 clinical trial of STA-1474 was performed in dogs with spontaneous tumors (145). This study demonstrated biologic activity in dogs with MCT, melanoma, OSA, and thyroid carcinoma and established a set of expected clinical toxicities, primarily consisting of anorexia, vomiting, and diarrhea, that were effectively managed with concomitant medications (145). Furthermore, HSP70 upregulation in both peripheral blood mononuclear cells and tumor tissues was established as a reliable biomarker of HSP90 inhibition. Lastly, objective responses to therapy were associated with sustained plasma levels of ganetespib of 200–600 ng/ml for 8–10 h. This association had not been predicted by the prior murine studies. Additional work was performed in dogs with MCTs to identify the optimal dose schedule of ganetespib, with the goal of determining a regimen that would provide sustained drug exposure (C. London, unpublished). Findings from this study recapitulated the observed dose/response relationship identified in the initial phase-1 study and demonstrated that administration of the drug over two consecutive days using a 1-h infusion protocol provided biologic activity equivalent to that achieved with an 8-h infusion given once per week. Ganetespib has since demonstrated activity in people with advanced non-small cell lung cancer, particularly in patients with *ALK* gene rearrangements, and clinical work in people is ongoing (146).

FUTURE ROLE OF DOGS WITH CANCER IN THERAPEUTIC DEVELOPMENT

Discovery

The twenty-first century has seen several marked successes in cancer drug development, including the approval of imatinib for the treatment of CML and ibrutinib for the treatment of CLL in people. Despite these successes, clinical development of new agents is a long and complex process, and setbacks are widespread. In an evaluation of 175 oncology drugs investigated from 1993 to 2002, half of the drugs that entered phase-3 clinical studies never achieved US regulatory approval (147). Reasons for drug failure are varied, including that the protein or pathway is not a valid anticancer target, the drug does not reach and/or bind to the intended target, the dose and/or regimen is incorrect, the PK/PD relationship is not fully understood, clinical toxicities are unacceptable, or the drug simply does not exhibit sufficient biologic activity (148). Importantly, the high rate of drug failure is simply not a sustainable business practice, as the costs associated with failure in the phase-2 and −3 setting range from $15 million to $40 million. This now translates into less than one drug approved per billion dollars of research and development invested. The incorporation of dogs with spontaneous cancers into this process has the potential to help mitigate some of the risk associated with cancer drug development by answering key questions early on that can facilitate triage of agents likely to fail and identify those agents with the most promising preclinical activity and safety (149).

Spontaneous models of cancer in companion animals contribute to discovery and early development of new agents in many ways. The biology and histology of cancers are shared between dogs and humans, with numerous genetic similarities that can be interrogated to identify disease-associated genes and therapeutic targets. Companion animals also share our

environment and thus are exposed to the same environmental risk factors. In many cases, therapeutic responses are comparable between dogs and humans, and the increased prevalence of certain cancers combined with rapid disease progression and early failure rate in dogs facilitates timely completion of clinical studies. Given the many hurdles in development of novel therapeutics, appropriately designed studies in dogs with spontaneous cancers can provide critical new information, including assessment of drug interactions and resistance patterns. Companion animals can be involved in clinical trials both before and after an IND has been filed.

Pre-IND Clinical Trial Work

Predictive preclinical models are an integral part of any IND application. Currently, such models typically involve the use of immunodeficient murine tumor xenografts; genetically engineered mice; and, more recently, patient-derived xenografts. However, none of these completely recapitulate the heterogeneity of spontaneous tumors and the complex interactions between the tumor, microenvironment, and immune system that are an integral part of spontaneous cancer in dogs. Information gained from clinical trials in dogs with cancer has the potential to provide critical new data, including the establishment of PD/PK relationships, definition of expected clinical toxicities, and evidence of biologic activity. Additionally, evidence of safety and activity in dogs with cancer can provide substantial support for an IND application, as was the case with the incorporation of data from verdinexor clinical trials into the selinexor IND application.

Laboratory dogs are commonly integrated into preclinical assessments of novel therapeutics, and the data generated are typically incorporated into an IND application. These data help to facilitate clinical trials in client-owned dogs with cancer, as dogs are the only species in which both laboratory-normal and spontaneous tumor-bearing patients can be used to assess novel therapeutics. This species-in-kind approach allows for the evaluation of agents in a phase-1/2 setting with rapid dose escalation, permitting the efficient achievement of maximum tolerated dose or other biologically relevant endpoints. In addition, evaluation of novel agents in healthy and clinically affected animals of the same species provides a better anticipation of expected adverse events, thereby derisking such studies in client-owned dogs.

Another major advantage of pre-IND clinical trial work in dogs relates to the population studied. In general, phase-1 studies of novel anticancer agents in people are performed in patients who have failed multiple therapies and have significant comorbidities. It is therefore possible that potential drug activity would be missed in this setting. Canine studies typically involve populations that are generally less heavily pretreated (or not treated at all) and have fewer comorbidities and thus may better represent a drug’s true therapeutic potential.

Post-IND Clinical Trial Work

After approval, postregistrational clinical studies are often conducted to gather information in support of additional therapeutic indications for the agent and to determine the potential activity of combination regimens (i.e., other therapeutic agents, additional treatment modalities). As in other phases of clinical drug development, leveraging the opportunities provided by the companion animal models can provide key information that answers

biologically important questions. For example, the post-IND approval studies with ganetespib in dogs helped to determine the appropriate treatment regimen that would provide an ideal level of drug exposure and demonstrated superior tumor response and target modulation using this regimen (C. London, unpublished). Post-IND studies in dogs with cancer can also be used to rapidly explore the activity of a novel therapy in the setting of microscopic metastatic disease, a task that is often difficult to perform in people with cancer given established standards of care. For example, toceranib was used in dogs with OSA following amputation and carboplatin chemotherapy to determine if a multitargeted small molecule inhibitor of VEGFR/PDGFR/KIT was capable of modulating the course of microscopic metastatic disease (150). This study showed no improvement in progression-free and overall survival with the inclusion of toceranib, and despite a negative outcome, it was used to inform the Children's Oncology Group and thereby to help guide future clinical studies in children with OSA.

CONCLUSIONS

Companion animals are uniquely positioned to improve our understanding of the biology and treatment of both human and canine cancers. Similarities in histology, biologic behavior, and molecular aberrations provide a solid foundation for comparative and translational oncology. As such, studying spontaneous cancer in dogs can provide important information that optimizes clinical drug development and informs subsequent studies in both human and veterinary medicine, ultimately leading to advancements in the care of people and dogs affected by cancer.

Glossary

Osteosarcoma (OSA)
malignant proliferation of osteoblasts; most common primary bone tumor in dogs

Melanoma
tumor arising from melanocytes; most common oral tumor in dogs

Transitional cell carcinoma (TCC)
malignant proliferation of the transitional epithelium lining the organs of the urothelial system; most common form of urinary bladder cancer in dogs

Linkage disequilibrium (LD)
in population genetics, the nonrandom association of alleles at different loci; i.e., the presence of statistical associations between alleles at different loci that are different from what would be expected if alleles were independently, randomly sampled based on their individual allele frequencies

Genome-wide association study (GWAS)
a study that evaluates genetic variants across the genome in individuals to examine genes associated with a particular disease

Mast cell tumor (MCT)

neoplastic proliferation of mast cells; most common cutaneous tumor in dogs

Histiocytic sarcoma
malignant proliferation of dendritic cells

Squamous cell carcinoma (SCC)
neoplastic proliferation of squamous epithelial cells

Comparative genomic hybridization (CGH)
molecular cytogenetic method of screening cells for detecting gains and losses in DNA (copy number variations)

Diffuse large B-cell lymphoma (DLBCL)
heterogeneous group of hematologic malignancies subdivided into germinal center B-cell (GCB) and activated B-cell (ABC) subtypes; most common lymphoproliferative disorder in dogs

Soft tissue sarcoma (STS)
heterogeneous population of mesenchymal tumors classified based on similar histopathologic appearance and clinical behavior

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